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JOURNAL OF ECONOMIC ENTOMOLOGY
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The Coinage of Common Names for Insecticides[1]

The need for standardized common names for pest control chemicals is well recognized, national and international organizations having been set up to promote their development. The problems involved in coining and standardizing common names are complex, however, particularly for proprietary compounds that are marketed in numerous countries, and therefore the development of common names sometimes lags far behind the need. The Entomological Society of America, through its Committee on Insecticide Terminology, endeavors to promote the development of common names for insecticides when the need becomes evident, and periodically publishes a list of the common names that have been approved for use in the publications of the Society.

The Society's Committee on Insecticide Terminology is represented on the American Standards Association's Committee on Common Names for Pest Control Chemicals, K62. Committee K62 considers proposed common names submitted by a sponsor and accompanied by the necessary fee; names that meet established requirements are accepted as American Standard common names. All American Standard common names of insecticides are placed on the Entomological Society of America's approved list. However, ASA Committee K62 does not initiate the coinage of common names; no matter how badly a common name for an insecticide may be needed, none will be considered by K62 unless proposed and supported by a sponsor.

ASA Committee K62 is represented on the International Organization for Standardization's Technical Committee ISO/TC 81, Common Names for Pesticides. Common names approved by ISO/TC 81 will also be placed on the Society's approved list if there is no conflict with a previously accepted common name.

The Society's Committee on Insecticide Terminology will also consider common names proposed directly to the Committee, and names that meet the established requirements will be added to the Society's approved list. Trademarks will be considered for acceptance as common names upon written notification that the trademarks have been released. To be acceptable, a common name must refer to the 100 percent pure pest control chemical, and must apply to a single compound. The common name need not be derived from the chemical name, and need not have chemical significance; commonly accepted chemical prefixes or suffixes may be used only in accordance with the standards of nomenclature of the American Chemical Society. The name should be short and must be distinctive, phonetically and in spelling, from the names of other chemicals. These requirements are uniform with those established by ASA Committee K62 and, insofar as possible, those of ISO/TC 81. Some common names accepted in the past have not met all these requirements; such names, if well established, will not necessarily be abandoned, but each will be considered on its individual merits.

The Committee urges the individuals or agencies most closely concerned with the production and use of specific insecticides to apply for common names, either to the Committee or, preferably, to ASA K62. Since patent holders are the most closely concerned with their proprietary compounds, and since their interests are usually world-wide, the Committee hopes they will assume the burden of developing common names for international use. The Committee itself will undertake the coinage of common names for inclusion in the Society's approved list only when the need is urgent and no sponsor has come forward.

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[1]Approved by action of the Governing Board, Entomological Society of America.

Fate of DDT in Larvicide Suspensions[1]

MALCOLM C. BOWMAN, FRED ACREE, JR., CLAUDE H. SCHMIDT, and MORTON BEROZA,
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ABSTRACT

The toxicity of DDT in aqueous-acetone suspensions to mosquito larvae is affected by the volume of suspension and the type of the test container. To learn whether the differences are due to settling, settling rates for different concentrations of DDT suspensions were determined. Settling does take place at the 1-p.p.m. level but not at 0.01 p.p.m., a concentration approximating that normally used in mosquito larvicide tests. In further studies of such suspensions, a high proportion of the DDT was found deposited on the inner walls of glass, aluminum, or paper containers when the liquid phase was withdrawn within minutes after preparation of the suspensions. The concentration of the DDT in suspension was thereby reduced. Another surprising finding was that more than 50% of the DDT from 0.01-p.p.m. suspensions was lost by volatilization with the liquid phase during 24 hours. This volatilization plus the deposition on the walls of the container appear to account for the loss of DDT from larvicide test suspensions. A physicochemical explanation for these findings is presented.

Considerable interest has centered about the factors influencing the toxicity of aqueous DDT suspensions in bioassays with mosquito larvae. Investigators have reported on the loss of DDT from such suspensions and have attributed this loss, at least in part, to settling. Cutkomp (1947) reported that dispersions of DDT at 1 p.p.m., aerated and aged for 2 hours, lost most of the DDT to the walls and bottom of the container, but that the DDT was not altered chemically. Krusé *et al.* (1952) implied that the variation in mortality obtained in their tests with *Anopheles quadrimaculatus* Say was due partly to the faster settling of DDT from suspensions in paper than in enamel or glass containers. They recognized also that the volume of suspension, and the surface area and type of container could cause variations. Hawkins & Kearns (1956) studied the effect of foreign ions on the settling of a 5-p.p.m. suspension and correlated the kill of mosquito larvae with the ionic charge about the sol particles (zeta potential) and the flocculation behavior of DDT from suspensions in glass, enamel, and paper containers.

That larval mortality is affected by the volume of the DDT suspension and the size and material of the container has recently been confirmed at the Orlando, Florida, laboratory by tests with three species of mosquitoes (Schmidt & Weidhaas 1959). During this investigation the question arose as to whether the settling rate in suspensions containing concentrations in the larvicide-test range (ca. 0.01 p.p.m.) might in fact be responsible for the difference observed in mortality, as had been suggested. Apropos of this point, Babers (1955) reported the solubility of DDT to be 0.0374 p.p.m. at 25° C. If DDT were soluble to this degree, no settling would be expected in the larvicide tests. However, no studies on the settling rate and the dispersion of DDT in suspensions containing such low concentrations have been reported.

These factors and others affecting the availability of DDT to mosquito larvae have now been determined through the use of radiolabeled DDT. The results of this investigation confirm the assumption of others that DDT settles from a suspension at 1 p.p.m., but no settling occurred at 0.01 p.p.m. The latter finding, which clearly shows that the loss of DDT in the larvicide test range could not be due to settling, prompted us to undertake a further investigation of this phenomenon, again utilizing the refined radioactive tracer technique.

The results were unexpected but not inexplicable. The DDT showed a surprising affinity for glass, aluminum, and paper surfaces. Within a few minutes after the suspension had been prepared in a container and the liquid portion removed therefrom, a high proportion of the insecticide was found deposited on the inner walls so that the concentration remaining in suspension was much below the original value. Another surprising result was that appreciable quantities of DDT from the 0.01-p.p.m. suspensions were lost by volatilization with the liquid phase over a 24-hour period. Actually this volatility of DDT approached the loss to be expected on the basis of the laws governing the "codistillation" (Rassow & Schultzky 1931) of compounds insoluble in water.

For convenience we have used the term "suspension" throughout this paper to refer to the DDT dispersions in water. It is recognized that the 0.01-p.p.m. concentration is roughly one-fourth the solubility value of DDT as reported by Babers (1955), so that the DDT might be considered as being in solution. We are not inclined to call them "solutions" because the concept of uniform distribution normally associated with this term is not in harmony with the unequal distribution of DDT found at this level.

The effect of these factors on the mortality of mosquito larvae will be discussed in another communication.

EXPERIMENTAL PROCEDURES.—Stock solutions of *p,p'*-DDT (m.p. 106–7° C.) and of *p,p'*-DDT-4-C^{14} (2.1 μc./mg.)[2] were made to contain 2, 20, and 200 μg./ml. in acetone that had been distilled after reflux over potassium permanganate. Suspensions were made in water that had been distilled over sulfuric acid-chromic acid, with both preparations of DDT at 1 and 0.1 p.p.m., but only C^{14}-DDT was used at 0.01 p.p.m. All tests were made at 25° ± 3° C., and a thermograph was used to obtain the range of temperature during the sedimentation experiments. Isooctane was purified by percolation through silicic acid until spectroscopically pure (Graff *et al.* 1944), and solutions of the residues in this solvent were analyzed for DDT in a Beckman DU spectrophotometer at 236 mμ. or by counting infinitely thin samples on stainless steel planchets in a Model PC-3 Nuclear proportional counter. These samples were plated by the

[1] Accepted for publication April 13, 1959.

[2] Kindly furnished by P. A. Dahm, of Iowa State College, and used by authority of the U. S. Atomic Energy Commission.

turntable technique from 250 μl. of solution. The residues of DDT recovered in the experiments with 0.01 p.p.m. were dissolved in 2 ml. of isooctane containing 16 μg. of unlabeled DDT to diminish the contamination of the counting chamber. The strong tendency of DDT in aqueous suspensions to deposit on glass made it necessary to rinse all sampling pipettes with isooctane to effect quantitative transfer. For the same reason suspensions were made up directly in the test containers.

Volatilization and Recovery of DDT from Suspension.—Although it was known that DDT can be extracted from suspension with hydrocarbon solvents, a method was desirable to minimize handling and losses at the low concentrations used. In preliminary experiments the acetone and water were removed from the suspension at 35 mm. Hg but the residue yielded only about 30% of the DDT. Even the residue obtained at 35 mm. Hg from an undried ether extract of the suspension did not yield all the DDT. This conclusion was surprising, because it was expected that DDT would be both stable and nonvolatile under these conditions. It was shown that the unaccounted-for DDT had actually volatilized. When 90 ml. was distilled at atmospheric pressure from 100 ml. of a 1-p.p.m. suspension, only a trace of DDT was detected in the residue. The DDT was recovered quantitatively from the distillate, and it had the same ultraviolet spectrum as the original DDT.

In the meantime it was found that 98% or more of the DDT could be recovered from 100 ml. of supension by extraction with three 25-ml. portions of isooctane, which dissolves only a trace of water, followed by removal of the solvent at 35 mm. Hg. This recovery procedure was used in all the experiments to be described. The entrainment of water in the isooctane extract must be avoided to prevent loss of the DDT.

Particle Size of DDT.—The settling rate of DDT in suspensions was determined in an Andreasen-type sedimentation apparatus (Loomis 1938) that was constructed from a 1-liter, graduated Pyrex cylinder and equipped with a 100-ml. sampling pipette that extended to the 200-ml. graduation. Five milliliters of the appropriate stock solution were added to 1 liter of water in the sedimentation apparatus and mixed by shaking for 1 minute. Aliquots (100 ml.) were withdrawn within 2 minutes and after settling intervals of 4, 24, and 48 hours. The amount of DDT recovered from each aliquot was used to calculate the quantity lost from suspension as based on the recovery from the 2-minute aliquot. The diameters of the DDT particles that corresponded to the settling intervals were calculated for this sedimentation system by means of Stokes' Law as follows:

$$d = 2\sqrt{\frac{9h\eta}{2(D_1 - D_2)gt}} \qquad (1)$$

where d is the diameter of the particle (cm.), D_1 and D_2 are the densities of the particle and the medium, respectively, g is the gravitation constant (cm./sec.^{2}), η the viscosity of the suspending medium (poises), h the distance between liquid surface and pipette tip when sample is withdrawn (cm.), and t the time from start of test (sec.). These values were then related to the quantities of DDT lost from suspension at the various settling intervals.

Dispersion of DDT in Glass, Aluminum, and Paper.—To 250 ml. of water in each of several pint glass jars (79 mm. dia.), 1.25 ml. of the appropriate stock solution was added and mixed by stirring with a glass rod for 1 minute. The distribution of DDT was determined within 2 minutes and after 24 or 72 hours. The samples for analysis consisted of the upper and the lower halves of the suspensions, which were removed by pipette, as well as the DDT that was removed from the inner surfaces of the jars by two 25-ml. rinses with isooctane. In some tests the exposed inner surfaces were divided into the upper one-third and the lower two-thirds plus the bottom.

The dispersion of DDT in pint aluminum measuring cups (90 mm. dia.) and pint ice-cream cartons (85 mm. dia.) was determined by the same technique except that the bottoms of the paper cartons were removed and analyzed separately. In order to diminish the interference caused by the impregnant in plating the extracts for radiometric analysis, the paper cartons were immersed in isooctane for 1 hour, rinsed, and dried at 105° C. before analysis.

RESULTS AND DISCUSSION.—These experiments show clearly that DDT volatilized with water from aqueous suspensions. Cutkomp (1947) reported losses of DDT from heated suspensions, which he attributed to thermal decomposition, but it now seems evident that his losses were due to codistillation of the DDT with water, because the conditions of some of our experiments were similar to his.

Babers (1955) concluded that DDT volatilized, because the background count increased when thin samples of C^{14}-DDT were counted in a proportional scaler. He was able to eliminate the background increase by adding the labeled DDT to approximately 1,000 parts of unlabeled DDT just prior to plating the samples for analysis. Although the plates were prepared by the removal of water, the volatility of DDT was not apparent owing to the high dilution factor. It is our belief that, when the radioactive DDT is not diluted and is plated from nonaqueous solutions, the background count increases because the counting chamber becomes contaminated with microcrystals of DDT removed mechanically from the plates during the flushing process.

During preliminary experiments on the particle size of DDT, aliquots were withdrawn within 2 minutes after mixing, the time required to adjust the apparatus and withdraw the sample. Analyses were made to establish the initial concentration of DDT in suspension, but the expected quantities were not obtained. However, a quantitative recovery was obtained when the sample for analysis was poured from the container while the suspension was being agitated. An investigation of this discrepancy revealed that 19 to 39% of the DDT, depending upon the concentration, was on the walls of the glass container. It is surprising that so much DDT was lost from suspension within a few minutes, although Cutkomp had recovered DDT from the walls of a container after 2 hours. This demonstrated the necessity for rinsing the sampling pipettes with isooctane.

The particle sizes of DDT in suspension are presented in table 1, which shows that at 1 p.p.m. almost 80% of the particles were less than 1.8 microns in diameter. Since it is necessary to make the usual assumptions for Stokes'

Table 1.—Per cent distribution of the particle sizes of DDT in aqueous suspensions determined by radioactive and ultraviolet analysis.

Settling Interval (Hours)	Diameter of Particles (Microns)	1 P.P.M. DDT Ultraviolet	1 P.P.M. C^{14}-DDT Radiometric	0.01 P.P.M. C^{14}-DDT Radiometric
0–4	>7.1	3.6	4.4	3.8
4–24	2.7–7.1	6.8	6.9	0.0
24–48	1.8–2.7	10.2	12.0	0.0
—	<1.8[a]	79.4	76.7	96.2

[a] Calculated by difference.

Table 3.—Per cent distribution of DDT in suspensions at 0.01 p.p.m. in glass, aluminum, and paper containers sampled within 2 minutes and after 24 hours.

Location of DDT	Glass 2 Min.	Glass 24 Hours	Aluminum 2 Min.	Aluminum 24 Hours	Paper 2 Min.	Paper 24 Hours
Upper half of suspension	31.7	18.0	19.6	5.3	37.5	1.0
Lower half of suspension	28.8	17.0	17.5	6.2	33.8	1.0
Upper third of exposed walls	—	—	49.2	29.3	—	—
Lower two-thirds of exposed walls and bottom	—	—	6.2	6.6	—	—
Exposed walls	—	—	—	—	17.6	14.9
Bottom	—	—	—	—	1.2	1.9
Walls and bottom	38.9	13.2	55.4	35.9	18.8	16.8
Total	99.4	48.2	92.5	47.4	90.1	18.8

Law and further assume that the concentration of DDT on the walls remained constant after 2 minutes, the calculated particle sizes should be regarded as approximations. However, the data show that DDT did settle at 1 p.p.m. On the other hand, negligible settling occurred at 0.01 p.p.m.; in fact, the small quantity that appeared to have settled in the first 4 hours might have been due to counting error.

Because of this information, the dispersion of DDT was studied at different concentrations and in different containers. The dispersion within 2 minutes at three concentrations in glass jars is shown in table 2. The pattern of distribution was essentially the same for the three concentrations of DDT or C^{14}-DDT, and comparable analyses were obtained with ultraviolet and radiometric techniques. More DDT was recovered from the upper than from the lower half of the suspensions. It is interesting to note that 39% to 56% was recovered from the walls of the containers, and this DDT might be unavailable to the larvae during a test.

The dispersion of DDT at 0.01 p.p.m. in different containers is shown in table 3. Within 2 minutes more DDT remained in suspension in the paper (71.3%) than in glass (60.5%) or aluminum (37.1%) containers. Most of the remaining DDT was found on the inner surfaces of the respective containers. After 24 hours more DDT was in suspension in the glass (35%) than in aluminum (11.5%) or paper (2%) containers. It is interesting to note, however, that during this time *more than 50% of the total DDT disappeared in these experiments.* The trend was for it to disappear faster from suspension in the paper than in aluminum or glass containers and faster from the inner surface of glass than from that of aluminum or paper containers. The loss from suspension correlated with the evaporation of water from the paper cartons (25 ml.), which was almost twice that from the glass or aluminum containers (15 ml.). This point will be discussed later.

Proof that DDT volatilized at room temperature with water (35 ml.) was obtained when less than 6% was recovered from open glass jars compared with quantitative recoveries from jars that had been closed during a 72-hour test period, as shown in table 4. Moreover, the increase on the lower inner surfaces of the closed jars proved that DDT settled from a suspension of 1 p.p.m. However, the data indicate that only negligible settling could have occurred in the closed suspensions of 0.01 p.p.m. These data together with those presented in table 3 show that most of the DDT immediately lost from suspension was deposited on the upper third of the container walls.

Ethanol is often used as the solvent for DDT in the preparation of mosquito larvicide suspensions. Although the data are not presented, it was found that the dispersion of DDT was essentially the same in aqueous suspensions containing ethanol or acetone.

A Rationale for the DDT Behavior.—The properties of DDT suspensions at concentrations as low as 0.01 p.p.m. have received little attention, because there is considerable difficulty in making the necessary quantitative measurements. However, if our concepts are oriented to accommodate the situation, the foregoing findings, although appearing odd at first glance, may be explained. For example, it is well known that hydrophobic (nonpolar) materials tend to accumulate more at the surface (interface) of water than in the bulk of the liquid. This phenomenon accounts for the lowering of the surface tension of water and has been verified experimentally many times. With extremely low concentrations, such as those encountered in our studies, it is natural to expect a large

Table 2.—Per cent distribution of DDT in glass jars within 2 minutes after preparation of suspensions determined by radiometric and ultraviolet analysis.

Location of DDT	1 P.P.M. DDT Ultraviolet	1 P.P.M. C^{14}-DDT Radiometric	0.1 P.P.M. DDT Ultraviolet	0.1 P.P.M. C^{14}-DDT Ultraviolet	0.1 P.P.M. C^{14}-DDT Radiometric	0.01 P.P.M. C^{14}-DDT Radiometric
Upper half of suspension	31.2	32.3	27.0	34.3	28.5	31.7
Lower half of suspension	28.5	31.7	20.5	23.5	21.1	28.8
Inner surface of jar	39.5	40.9	56.3	48.2	51.2	38.9
Total	99.2	104.9	103.8	106.3	100.6	99.4

Table 4.—Per cent distribution of DDT in aqueous suspensions in open and closed glass jars, sampled within 2 minutes and after standing 72 hours.

Location of DDT	1 P.P.M.			0.01 P.P.M.		
	2 Min.	72 Hours		2 Min.	72 Hours	
		Closed	Open		Closed	Open
Upper half of suspension	35.3	20.2	5.1	31.7	37.6	2.8
Lower half of suspension	32.0	19.9	9.8	28.8	34.8	2.0
Upper third of exposed walls	22.1	21.8	10.3	—	18.8	0.5
Lower two-thirds of exposed walls plus bottom	5.1	36.9	43.8	—	11.4	.2
Walls and bottom	27.2	58.7	54.1	38.9	30.2	.7
Total	94.5	98.8	69.0	99.4	102.6	5.5

proportion of the nonpolar solute to be located at the interface—that is, at the walls and at the upper surface of the liquid. Some may prefer to regard the DDT as being so hydrophobic that it tends to get out of the body of the liquid, where it is surrounded on all sides, and migrate to the interface, where it is only partially in contact with water and partially with another substance, such as air, aluminum, glass, or paper. The affinity of DDT toward the interface is a real force, as judged by the large proportion deposited on the walls of the container within a few minutes after the suspension is prepared and then withdrawn. Much of the DDT clings to the surface of the container rather than remaining in suspension. It is our belief that this is a logical explanation for the deposition of DDT on the walls of the container.

The second supposedly odd finding is the rapid volatilization of DDT with the concurrent vaporization of water or with steam. DDT is usually considered nonvolatile with steam; but, when very low concentrations are dealt with, the volatility becomes explicable not only qualitatively but semi-quantitatively as well.

With compounds insoluble in water the vapor pressure of an immiscible phase is known to be substantially unchanged by the presence of water. The general equation relating the volatility of two materials (A and B) to molecular weight and vapor pressure (Glasstone 1946) is:

$$\frac{W_A}{W_B} = \frac{M_A P_A}{M_B P_B} \qquad (2)$$

where W is the weight of distillate, M the molecular weight, and P the vapor pressure. By letting A represent DDT and B water in this equation, the vapor pressure of DDT (P_A) at 25° ± 3° C. was calculated from the amount of water and DDT volatilized from the four 0.01-p.p.m. suspensions indicated in tables 3 and 4. The data and results follow:

Container	Grams Volatilized		DDT Volatilized (%)	Calculated Vapor Pressure of DDT (mm. Hg)
	Water	DDT		
Table 3:				
Glass	15	1.30×10^{-6}	51.8	1.1×10^{-7}
Aluminum	15	1.32×10^{-6}	52.6	1.1×10^{-7}
Paper	25	2.03×10^{-6}	81.2	1.0×10^{-7}
Table 4:				
Glass	35	2.36×10^{-6}	94.5	0.8×10^{-7}

For comparison, the following vapor pressures of DDT in the temperature range of our experiments were calculated from the data reported by Balson (1947):

2.1×10^{-7} mm. Hg at 22° C.
3.4×10^{-7} mm. Hg at 25° C.
5.4×10^{-7} mm. Hg at 28° C.

The approach of the calculated vapor-pressure values in our experiments at 25° C. to Balson's (maximum) value, or, otherwise stated, the facile codistillation of DDT with water at this temperature, is an excellent indication that the DDT concentrates at the air-water interface of the 0.01-p.p.m. suspensions. It is possible that some of the DDT deposited on the upper third of the container wall results from the DDT at the air-water interface clinging to the wall on withdrawal of the suspension for analysis.

If Balson's vapor pressure of DDT were substituted in equation (2), the calculated amount of DDT would be the maximum that could codistil with the given quantity of water. Maximum distillation could be achieved only if excess DDT, as evidenced by two immiscible phases, were present throughout the vaporization period. This point was verified when 100 ml. of a 250-p.p.m. suspension (agglomeration occurred immediately) was subjected to distillation at atmospheric pressure (765 mm.) with the following results:

Water distilled (ml.)	*DDT distilled (mg.)*
19.5	1.708
40.3	3.506

The vapor pressure of DDT at 100° C. was calculated from these data to be 3.38×10^{-3} mm., which agrees very well with 4.8×10^{-3} mm. obtained by Balson.

On the other hand, the codistillation rate of DDT with water would be expected to decrease as the concentration was reduced below saturation. This point was verified when 100 ml. of a 25-p.p.m. suspension (2.5 mg. DDT, suspension turbid) was distilled at atmospheric pressure (765 mm.) with the following results:

Water Distilled (ml.)	*DDT Distilled (mg.)*
18.7	1.413
18.7	1.777
40.2	1.961
54.2	2.054
83.2	2.207 (88.3%)

Analyses showed that 0.093 and 0.114 mg. of DDT remained in the suspension and on the walls of the still, respectively. The total DDT recovered was 2.414 mg. (96.6%). During this distillation no visible agglomeration occurred and turbidity disappeared after 25 to 30 ml. of water had distilled.

The codistillation of DDT with water in these experiments with 25 and 250 p.p.m. are compared in figure 1. When two phases were present in the 25-p.p.m. distillation as evidenced by turbidity, the codistillation rate of DDT closely approached that of the 250-p.p.m. distillation during which two phases were present. When the 25-p.p.m. suspension cleared, excess DDT was no longer present and the codistillation rate dropped below the maximum value. These results parallel our findings at 25° C. but are not strictly comparable because of the agitation obtained at ebullition.

Examination of some of our other data on the volatility with steam of DDT at 1 p.p.m. in 100 ml. of suspension at atmospheric pressure is worthwhile. When Balson's vapor pressure of DDT at 100° C. (4.8×10^{-3} mm.) plus

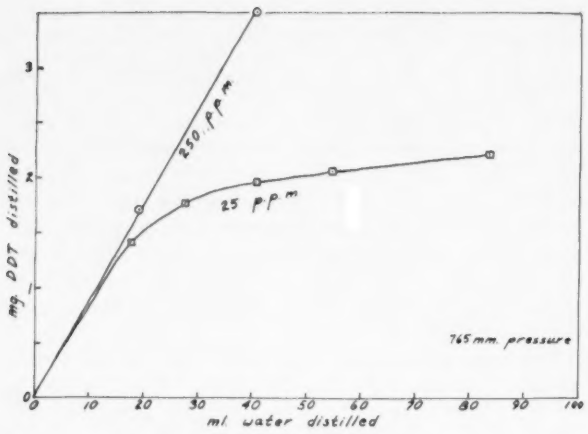

FIG. 1.—Codistillation of DDT with water.

other known data are substituted in equation (2) and we assume that the theoretical (maximum) amount of DDT codistils with water, then the weight of water that has to be distilled to carry over all the DDT is less than 1 gram. Naturally, when 90 ml. was distilled, no DDT was found in the remaining 10 ml.

Since it is established that the codistillation of DDT is appreciable, it is significant to note that small changes in temperature produce a large change in DDT volatility; *e.g.*, at 28° C. the vapor pressures of DDT and water are roughly 2.5 and 1.4 times those at 22°, therefore, DDT will codistil about 3.5 times faster at 28° than at 22°. For this reason, the control of temperature in the larval tests should be precise. Humidity and air movement will also affect the volatility of water and therefore of DDT. Probably the simplest means of controlling these variables is to prevent volatilization by closing the test container. Compounds having solubilities in water much greater than DDT would not be expected to behave the same as DDT, *i.e.*, codistil and show marked boundary effects. These points have been verified by biological tests to be reported in another communication.

CONCLUSION.—Although a change in concentration of DDT in simulated larvicide tests has been substantiated, at the 0.01-p.p.m. level DDT in aqueous suspensions probably becomes unavailable to mosquito larvae because of volatilization and its affinity for liquid boundaries rather than settling from suspension, as proposed by previous investigators.

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The Effect of Late-Season Applications of Insecticides on Diapausing Boll Weevils[1]

J. R. BRAZZEL[2]

ABSTRACT

Three insecticides were applied to cotton plants from August 22 through November 20, after the normal control program was completed, to determine the effect on diapausing boll weevil (*Anthonomus grandis* Boh.) populations under caged conditions. Determinations by dissection indicated relatively large numbers of weevils diapaused in the toxaphene and calcium arsenate treatments but only one diapaused in the plots treated with methyl parathion. These results indicated that properly timed late-season insecticide applications, coordinated with cultural practices, may be effective in reduction of the overwintering boll weevil population.

The importance of the overwintering population of the boll weevil, *Anthonomus grandis* Boh., has been recognized for more than 50 years. Control programs practiced before the discovery of effective insecticides were based to a great extent on cultural practices designed to decrease the overwintering population of the weevil. Hunter & Hinds (1904) advocated destruction of the cotton plants as soon as possible after harvest in the fall to decrease the number of weevils that would survive the winter. Hinds (1908) considered the time between the maturity of the cotton crop and the first frost as the strategic period for reducing the number of weevils which may survive hibernation and attack the crop the following season. Newell & Paulsen (1908) reported on experiments designed to reduce weevil damage by spraying cotton fields in the fall to destroy the foliage and squares. These authors stated that the most important step in the production of a cotton crop was to reduce the number of overwintering weevils.

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After the discovery of efficient boll weevil insecticides, the cultural control practices received less attention. However, the desirability of reducing overwintering weevil populations is still recognized. Walker & Hopkins (1956) reported considerable reductions in the overwintered weevil population by application of granulated insecticides to hibernation quarters during March. In some areas insecticide applications on cotton are made early in the growing period to control the overwintered weevils.

Recent work reported by Brazzel & Newsom (1959) indicated that in the Temperate regions the boll weevil survives the winter months in a state of diapause. Diapausing weevils occur in the field in greatest numbers during a period from the beginning of maturity of the cotton crop until frost kills the plants. Also, preliminary results indicate that a period of approximately 3 weeks or longer is required for a weevil to feed and accumulate sufficient fat reserves to attain a firm state of diapause. This information indicates that any chemical and cultural measures that decreased the weevil population and reduced the food supply during the period in which diapause occurs would materially reduce the overwintering boll weevil population. The research reported herein was designed to evaluate the effect of three different insecticides on diapausing weevils when applied during the cotton harvest season.

METHODS.—In order to isolate the boll weevil populations used in this experiment the test plots of 1/200th acre were covered with plastic screen cages. Treatments included toxaphene at 3 pounds per acre, calcium arsenate at 10 pounds per acre, methyl parathion at 0.5 pounds per acre and an untreated check. Each treatment was replicated four times in a randomized block design. The weevil infestation was obtained in the caged plots by introducing three males and three females into each cage on June 19 after the cotton had started fruiting. These weevils were field-collected first-generation individuals.

The diapausing status of the populations was determined by periodically dissecting a sample of weevils from each treatment. Criteria used to distinguish diapausing weevils were described by Brazzel & Newsom (1959) as follows: Female—Ovaries atrophied to approximately 2.0 mm. in length from the vaginal pouch to the tip of the ovarioles, no eggs visible in the ducts with 9× magnification and visible fat deposits. Male—Testes atrophied to approximately 0.6 mm. in diameter, yellowish, no sperm bundles present, and visible fat deposits.

In order to obtain sufficient numbers of weevils in the sample collections, insecticide applications were not started until approximately 50% of the squares were damaged. Insecticide treatments were made on approximately a 5-day schedule from July 10 through August 11. Thereafter, applications were made on a 10- to 12-day schedule until November 20, except that the August 11, 22, September 2, 24, October 17, and November 1 applications of calcium arsenate were not made.

Beginning July 21 weevil collections were made at weekly intervals until August 11. Thereafter, collections were made immediately prior to each insecticide application. Weevils were collected from each cage of a treatment and pooled. Insofar as possible a total of 40 weevils, 20 males and 20 females, were dissected from each sample to determine the number of diapausing individuals in each treatment.

The test was terminated on November 20, a week prior to the first frost. The cotton plants were cut at the ground level and removed from the cages. The layer of debris on the soil in each cage was collected and sifted through a series of screens to determine the number of diapausing weevils in the trash.

RESULTS AND DISCUSSION. The boll weevil infestations in the treatments are shown in table 1. More than 50% of the squares were punctured in all treatments by July 15 and the infestation remained high throughout the duration of the test except for a few small numbers recorded in the calcium arsenate treatment. Under the conditions of this test calcium arsenate was so effective for boll weevil control that applications were omitted to allow the population to build up sufficiently to obtain weevil samples.

The percentage of diapausing weevils found in the treatments from July 21 through November 20 is presented graphically in figure 1. The first diapausing weevils were found in the July 28 collection with 5% and 10% in the toxaphene and untreated check, respectively. Diapausing weevils were present in all subsequent collections from these two treatments. One weevil in an advanced state of diapause was found in the August 22 collection from the methyl parathion plots. Several calcium arsenate applications were omitted but diapausing weevils were found in the October 6 and subsequent collections from these plots.

Diapausing weevils increased rapidly to about 40% on August 4 in the untreated check. The percentage of the population in diapause varied from 15% to 40% until November 10, when it reached 52%. The same pattern was found in the weevil population of an untreated field nearby, indicating that the caged conditions under which this test was conducted had little, if any, effect on the occurrence of diapause in the weevil population.

The percentage of diapausing weevils in the toxaphene treatment was smaller but followed approximately the same pattern as the untreated check. The number of diapausing individuals in the toxaphene treatment was about one-half that of the check except for the collections made in late October and November, when they approached the same level.

Table 1.—Percentage of squares damaged by the boll weevil.

DATE	TREATMENT			
	Untreated Check	Toxaphene	Calcium Arsenate	Methyl Parathion
June 20	17.7	5.8	6.8	8.5
25	27.8	21.5	25.5	24.8
30	52.5	36.0	37.3	38.8
July 4	63.0	37.3	35.5	36.8
15	81.8	82.5	70.5	66.5
21	90.0	88.3	81.3	77.5
28	98.3	73.5	52.0	69.0
Aug. 4	100.0	94.0	31.5	60.5
Sept. 2	77.5	73.5	69.8	50.8
12	89.8	77.0	56.6	59.0

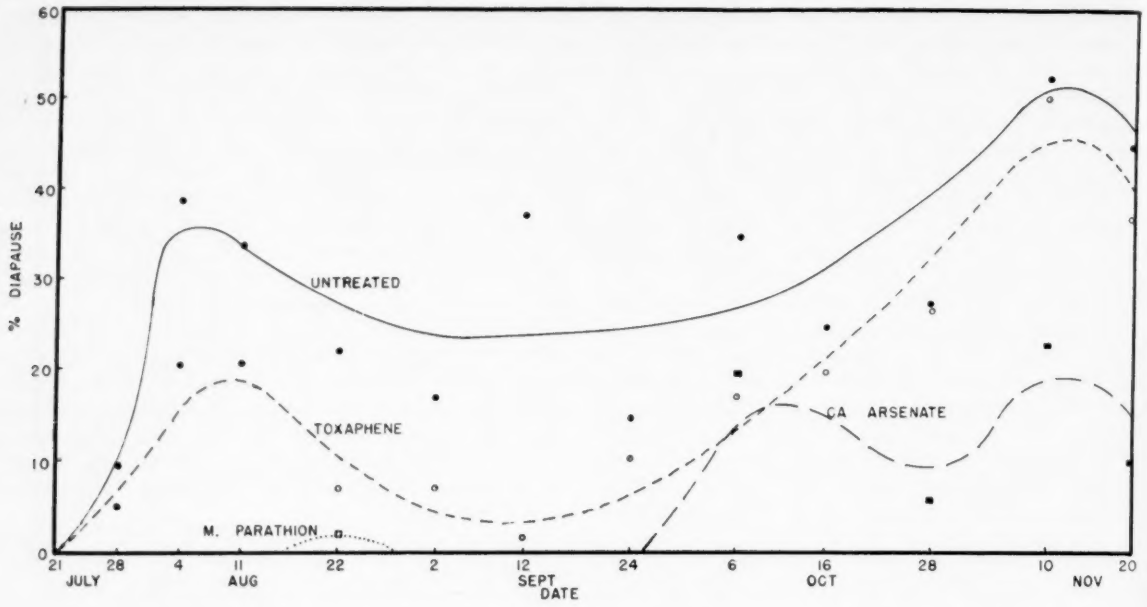

FIG. 1.—Percentage of diapausing boll weevils found during the season in plots treated with toxaphene, calcium arsenate, and methyl parathion.

The calcium arsenate treatment was so effective under the caged conditions that the application schedule had to be decreased in order to keep an adequate weevil population for sampling. Weevils were extremely difficult to find in some collections and 25% to 85% of the weevils collected from July 21 through September 24 were less than 3 days of age. Although diapausing weevils occurred late in the season in the calcium arsenate treatment, these data indicate that regular applications of this insecticide during the cotton-growing season would reduce the numbers of weevils that attained the diapausing condition.

The fact that only one diapausing weevil was found in the methyl parathion treatment indicated that this insecticide was quite effective in reducing the numbers of weevils that go into diapause.

Examination of the ground trash collected from each treatment indicated 2,200 diapausing weevils per acre in the untreated check but none in any of the plots treated with insecticides. This fact was surprising in view of the fact that large numbers of weevils in the toxaphene and calcium arsenate treatments appeared to be in an advanced state of diapause and were not visibly different from those in the same state in the untreated plots.

Results of this test indicate that application of methyl parathion at 10- to 12-day intervals during the harvest period will materially reduce the numbers of weevils that go into diapause, thereby reducing the overwintering weevil population. The results obtained with this insecticide are not considered to be a case of the insecticide's preventing diapause from occurring in the weevil population. Previous research has shown that organophosphorus insecticides are as effective for control of "fat" boll weevils as for "lean" ones, but the fat weevils, compared with lean ones, may be up to 100 times more tolerant to the chlorinated hydrocarbon insecticides. One of the first manifestations of diapause in a weevil population is an increase in fat content. Thus, a population in which diapausing weevils were present would be difficult to control with chlorinated hydrocarbon insecticides. In fact, treatments with this class of insecticides would be effective for only the nondiapausing weevils.

Preliminary laboratory data indicate that it takes 3 weeks or longer after emergence from a square for a weevil to attain firm diapause and move into hibernation quarters. A 10-day schedule of insecticide applications would result in each diapausing weevil's being subjected to two insecticide applications. Therefore, it appears that the weevils were not prevented from going into diapause, but that they were killed by the insecticide before they could attain this condition.

It seems likely that any organophosphorus insecticide that is effective for boll-weevil control would be satisfactory for this program. Methyl parathion was selected for use in the present test because of its short residual properties. This feature would be an advantage because of the fact that applications would be made during the cotton harvest period.

The 10- to 12-day schedule of applications was started in this test on August 22 and continued through November 20 in order to give the weevils every opportunity to diapause during the fall. This schedule resulted in the use of nine treatments. The treatments made from July 10 through August 11 were considered necessary for normal weevil control while the cotton crop was being produced. It would be possible to put this program into practice with fewer insecticide applications than the number used in this test. Two or three insecticide applications and the use of efficient defoliants or desiccants to destroy the food supply until stalks were destroyed mechanically or killed by frost should produce the same results. However, if defoliation or desiccation did not eliminate all the food supply of the weevil, insecticide applications would be necessary until the stalks were destroyed.

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The Use of Systemic Insecticides for Control of the Cabbage Aphid on Cabbage and Cauliflower[1]

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ABSTRACT

The systemic insecticides Thimet®, (*O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate), Bayer 19639 (Di-Syston® =*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate), demeton thiol-isomer, (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorothioate) and demeton were evaluated in a series of field experiments for control of the cabbage aphid, *Brevicoryne brassicae* (L.), on cabbage in southern California. Seed-coat applications of both Bayer 19639 and Thimet on activated charcoal at the rate of 4 pounds of toxicant per 100 pounds of seed caused a reduction in plant stand. Moderate control of the green peach aphid, *Myzus persicae* (Sulz.), was obtained for as long as one month by this method.

Granulated Bayer 19639 and Thimet applied to the seed furrow at the rate of about 1 pound of toxicant per acre gave control of the cabbage aphid for 2 months with no apparent reduction in the number of plants. Bayer 19639 applied at about the same rate as a spray to the seed furrow also gave aphid control for 2 months. Thimet and Bayer 19639 were applied as granules at the rate of ½, 1, and 2 pounds per acre to the transplant furrow. Bayer 19639 at the 1- and 2-pound dosages gave good control for at least 2 months under moderate population pressure. Thimet at similar dosages gave good initial kill but was not effective over as long a period. Both materials at one-half pound per acre gave early control but lost their effectiveness after about 1 month. Bayer 19639 applied as granules directly to aphid-infested foliage at dosages as low as one-fourth pound per acre gave good control in 5 days and was still effective 17 days after treatment. Thimet at similar concentrations was also effective. Bayer 19639 generally gave longer aphid control than Thimet.

Residue studies indicate that Thimet and Bayer 19639 applied to the plants at time of transplanting, or soon thereafter, had dissipated almost entirely from the cabbage heads at harvest time. Samples also indicate that Thimet breaks down somewhat faster than Bayer 19639.

Systemic insecticides have been used with widespread success in the control of various plant-sucking pests and have been demonstrated to have a limited effect on certain chewing insects (Ashdown & Cordner 1952, Ivy *et al.* 1954, Parencia *et al.* 1957, Reynolds *et al.* 1957). Materials of this type are especially noted for their effectiveness on pests relatively inaccessible to the more conventional contact poisons. The cabbage aphid, *Brevicoryne brassicae* (L.), owing to its habit of feeding on the underside of young unfolded leaves of small cabbage plants, often stunting and curling the foliage, can be placed in this group. At present one of the most successful means of controlling this aphid is by the application of demeton as a foliage spray. Recently, however, formulation methods have made available various nonliquid carriers impregnated with systemic insecticides which can be applied directly on or adjacent to selected portions of the plant and still retain their systemic qualities. The increased versatility of these formulations greatly enhances the selectivity of the insecticides and warrants testing on a variety of crops and pests.

MATERIALS AND METHODS.—In nine field experiments applied for control of the cabbage aphid on cabbage and cauliflower, the following materials and formulations were used: (1) Bayer 19639 (Di-Syston® = *O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate)—50% on charcoal, 2% and 2½% on granules, and 25% emulsion concentrate; (2) Thimet® (*O,O*-diethyl *S*-(ethylthio) methyl phosphorodithioate)—44% on charcoal, 2% on granules, and 25% emulsion concentrate; (3) demeton thiol-isomer (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorothioate)—1% on granules; and (4) demeton—25% emulsion concentrate.

Applications were made at seeding time, at transplant time, at several intervals after transplanting, and to maturing plants. All treatment plots were one row wide and replicated four times unless otherwise noted.

Seed-coat applications were made with charcoal formulations of Thimet and Bayer 19639 (8 grams of formulated material, 13 to 16 cc. of water, 0.3 grams of methyl cellulose sticker, plus 100 grams of cabbage seed) just prior to planting.

The granulated materials were applied manually to the soil in a 2- to 2½-inch band and were covered with a ½- to 1-inch layer of soil. Foliar applications were made with a hand-operated rotary-type duster with a single nozzle, or by sprinkling the material on the plants by hand. Because of variation in row width from field to field the dosages were based on the number of grams of formulated materials per running foot of row. Thus, 52 grams of a 2% formulation per 50 feet of row is the equivalent of 1 pound of active ingredient per acre calculated on the basis of a 2-foot row width.

[1] Paper No. 1142, University of California Citrus Experiment Station, Riverside, California. Accepted for publication April 9, 1959.

Table 1.—Effect of systemic insecticides on aphid populations on cabbage plants.[a] 1957.

Treatment	Material	Toxicant per Acre (Oz.)	No. of Plants/ 200 Feet March 13	Results per 20 Plants Examined			
				March 22[b]		April 11	
				Number of		Number of	
				Aphids	Infested Plants	Aphids	Infested Plants
Seed coat[c]	Thimet (44%)	—	308	86	15	277	20
	Bayer 19639 (50%)	—	490	49	19	449	20
Granules	Bayer 19639 (2%)	15.0	729	6	4	18	10
	Thimet (2%)	15.0	764	16	8	78[d]	8[d]
Spray	Bayer 19639 (25% EC)	18.8	720	10	6	24	13
Untreated	—	—	779	108	18	231	20
L.S.D. at 5% level			183.4	16.2	4.4	126.3	ns

[a] Materials applied February 4 as seed coatings and as granulated and spray formulations in seed furrows.
[b] Counts made on the green peach aphid, *Myzus persicae* (Sulz.), on the basis of 80 plants per treatment.
[c] Eight grams of formulated material, 13 to 16 cc. of water, 0.3 grams of methyl cellulose sticker per 100 grams of cabbage seed.
[d] Two replicates, plants stunted in size, with no aphids. Figures not included in calculation of L.S.D.

Where a natural infestation of aphids failed to develop, the plants were artificially infested either by brushing aphids into the crowns of the plants or by placing the aphids in a small cage on a leaf for a period of 3 to 7 days and recording the subsequent mortality.

Treatment at Seeding Time.—*Test No. 1.*—On February 4, 1957, treated and untreated seeds were placed in open seed furrows with a hand seeder. Granulated Thimet and Bayer 19639 were sprinkled over the untreated seeds, and liquid Bayer 19639 was also applied over untreated seeds as a spray at the rate of 70 gallons per acre, using a hand sprayer with a single flat fan-type nozzle. The seeds were covered with soil immediately after being treated. Aphids were caged on five plants in each of the four replicates 60 days after application, and the survivors were counted 7 days later (April 11). The results of this test are given in table 1.

On March 13 (37 days after treatment) the seedlings were ½ to 2 inches high. The plants in the Thimet-treated plots were slightly smaller than the others, and both seed treatments had significantly reduced germination. On March 22 (46 days after treatment) the plants had two to five true leaves and were 2 to 2½ inches high. The plants in the plots treated with granulated Thimet were still slightly stunted, but plants grown from the Thimet-coated seed, although reduced in number, were equal in size to the plants of the other treatments on this date.

As shown in table 1, the plants in the plots of both seed treatments had significantly fewer aphids than the untreated plants. The reductions were not considered satisfactory, however, and were significantly poorer than the other methods of treatment. On April 11, neither seed treatment was better than the untreated. The granulated Thimet and Bayer 19639 and the spray treatment of the latter compound were still giving good aphid kill 2 months after application. However, aphids placed on the plants May 4 and counted 10 days later indicated that all treatments had lost their effectiveness at this time.

Test No. 2.—On April 1, 1957, untreated seed was planted in seed furrows treated at the rate of 1 pound per acre with granulated Thimet, Bayer 19639, and demeton thiol-isomer. Each treatment was replicated only twice. Cabbage aphids were caged on the young plants 28 and 35 days after treatment; the survivors were recorded 3 days later. The results of this test appear in table 2.

Neither phytotoxic effects nor significant differences in germination were observed. The treatments were giving good control 31 days after application, but after 38 days only Bayer 19639 was maintaining control. A count of aphids caged on the leaves 59 days after treatment indicated that aphid kill was erratic and that no material was giving effective control at this time.

Treatment of Transplants.—*Test No. 3.*—On February 11, 1957, demeton, Bayer 19639, and Thimet solutions were poured into trenches around the base of young transplants at the rate of 50 cc. per plant. Young cabbage plants were also planted directly into transplant furrows treated at the rate of 1 pound of toxicant per acre with Thimet, Bayer 19639, and demeton thiol-isomer granules. In addition, Thimet and Bayer 19639 granules were side dressed at a similar rate per acre on the irrigation furrow side of the row 2 inches from the plants 2 weeks after transplanting. The plots were irrigated the day after the transplanting. Forty-two days after treatment the plants were artificially infested with cabbage

Table 2.—Effect of granulated systemic insecticides on cabbage aphid populations on cabbage plants.[a]

Material	Toxicant per Acre (Lb.)	No. Plants/ 18 Feet, April 16[b]	Per Cent Aphid Reduction Under Untreated Plants	
			May 2	May 9
Thimet (2%)	1	529	98	78
Bayer 19639 (2%)	1	522	80	88
Demeton thiolisomer (1%)	1	588	98	69
Untreated	—	602	—	—
L.S.D. at 5% level		ns		

[a] Materials applied in seed furrow on April 1, 1957.
[b] Plants 1 to 1½ inches high. No phytotoxic effects observed.

Table 3.—Effect of systemic insecticides on cabbage aphid populations on cabbage plants.[a]

Material		Toxicant per Acre (Lb.)	Results per 20 Plants Examined: March 7[b] Number of Infested Plants	March 7[b] Number of Aphids	April 3 Number of Infested Plants	April 3 Number of Aphids
Solutions poured around base of plants						
Demeton	(0.25%)	8.8	0	0	0	0
	(0.125%)	4.4	0	0	1	5
	(0.0625%)	2.2	0	0	9	95
Thimet	(0.25%)	8.8	0	0	1	2
Bayer 19639	(0.25%)	8.8	0	0	0	0
Granules applied in transplant furrow prior to transplanting						
Thimet	(2%)	1.0	0.36	2	14	171
Bayer 19639	(2%)	1.0	0	0	5	26
Demeton thiol-isomer	(1%)	1.0	0	0	15	172
Granules applied as side dressing						
Bayer 19639[c]	(2%)	1.0	0.40	0.5	10	68
Thimet[c]	(2%)	1.0	0	0	19	340
Untreated	—	—	4.0	120	19	415
L.S.D. at 5% level			—	—	5.1	128.2

[a] Materials applied by various methods to the soil at time of transplanting February 11, 1957.
[b] Based on one replicate.
[c] Side-dressed 2 inches from plants February 25.

aphids to supplement the natural infestation present. The results of this test are shown in table 3.

A plant-stand count on March 7 (24 days after treatment) gave some indication that the Bayer 19639 solution caused a slight reduction in plant stand. On March 7 all treatments gave good aphid control. Within 51 days after the original soil application, all treatments except the Thimet side dressing had significantly reduced the aphid population. At this time the plants were 6 to 8 inches high and beginning to head. A final observation 78 days after treatment indicated that none of the Thimet and Bayer 19639 granules, applied at 1 pound to the acre, were giving control.

Test No. 4.—On April 14, 1957, young cabbage plants were placed in transplant furrows treated with Bayer 19639, Thimet, and demeton thiol-isomer granules. Both Bayer 19639 and Thimet were applied at 0.5, 1.0, and 2.0 pounds of toxicant per acre, but demeton thiol-isomer was used only at the highest rate. The granules had been placed in the soil 3 days after irrigation and 9 days preceding transplanting. Aphids were caged on five

Table 4.—Effect of granulated systemic insecticides on cabbage aphid populations on cabbage.[a]

Material		Toxicant per Acre (Lb.)	Per Cent Aphid Reduction on Counting Date[b] April 29	May 6	June 3
Bayer 19639	(2%)	2.0	100	100	94
		1.0	100	99	95
		0.5	100	98	42
Thimet	(2%)	2.0	100	99	68
		1.0	100	100	0
		0.5	100	90	11
Demeton thiol-isomer	(1%)	2.0	95	30	10

[a] Materials applied to transplant furrow 9 days before transplanting on April 14, 1957.
[b] Untreated plots had a total of 90, 360, and 292 aphids per 20 plants, respectively, on the three counting dates.

plants per treatment on April 29, and on 20 plants per treatment on May 6 and 27 (15, 21, and 42 days, respectively, after transplanting). The results of this test appear in table 4.

There was no apparent plant injury following this method of treatment and the indicated dosages. Fifteen days after transplanting, all the treatments were giving excellent control, and only the demeton thiol-isomer was poor 21 days after transplanting. On the final count, 42 days after transplanting, the 1- and 2-pound dosages of Bayer 19639 were the only treatments giving excellent control. At this time, Thimet at 2 pounds per acre was still fair, and the other treatments had lost their effectiveness. Observations indicate that there was less injury from cabbage looper, *Trichoplusia ni* (Hbn.), in the high Thimet dosage plots, and is apparently due to some mortality among the first-instar larvae.

Test No. 5.—On August 16, 1957, Bayer 19639 and Thimet granules plus a Thimet solution were side dressed at approximately 1 pound of toxicant per acre 2 inches from the base of young cabbage plants several days after transplanting. The Thimet solution was poured in a continuous stream along the furrow at the rate of 130 cc. per 50 feet. The plants were not irrigated until at least 2 weeks after treatment. The results of this test are given in table 5.

Twenty-six days after treatment the Bayer 19639 granules and to a lesser extent the Thimet solution were giving significant control of the cabbage aphid. However,

Table 5.—Effect of systemic insecticides on cabbage aphid populations on cabbage.[a]

Treatment	Material		Toxicant per Acre (Lb.)	Results per 40 Plants Examined: August 30[b] Number of Aphids	August 30[b] Number of Infested Plants	September 11 Number of Aphids	September 11 Number of Infested Plants
Granules	Bayer 19639	(2%)	1	22	4	63	10
	Thimet	(2%)	1	380	14	289	22
Solution	Thimet	(25%)	0.96	94	11	112	17
Untreated			—	136	12	449	22
L.S.D. at 5% level				ns	ns	227.1	ns

[a] Granules and solutions applied as side dressings 2 inches from the plant August 16, 1957.
[b] Plants not irrigated for at least 2 weeks after treatment date.

Table 6.—Effect of granulated systemic insecticides on cabbage aphid populations on cabbage.[a]

Material	Toxicant per Acre (Lb.)	Results per 40 Plants Examined			
		August 21[b]		September 5[b]	
		Number of		Number of	
		Aphids	Infested Plants	Aphids	Infested Plants
Bayer 19639	2	88	21	289	25
Thimet	2	145	25	683	37
Untreated	—	881	40	2,015	39

[a] Materials applied to transplant furrows August 7, 1957 just prior to transplanting.
[b] Heavy migration of winged aphids into plot area from nearby infested cabbage plants.

in this particular test the aphid counts were somewhat more erratic than usual. This was perhaps due in part to the extended interval between treatment and irrigation; thus possibly less material reached the root zone to be picked up by the plants.

Test No. 6.—On August 7, 1957, young cabbage plants were transplanted into soil treated with Bayer 19639 and Thimet granules at the rate of 2 pounds of toxicant per acre, and irrigated immediately afterward. The results of this test are presented in table 6.

A steady migration of winged aphids took place into the plot area from nearby heavily infested cabbage plants. Fifty-four days after treatment all plants were infested; however, the plants treated with Bayer 19639 showed the least amount of aphid injury. At this time, the Bayer 19639 and Thimet plots had 86% and 66%, respectively, less aphids than the check plot.

Application of Granules to Foliage.—*Test No. 7.*—On July 2, 1958, granulated Bayer 19639 (2½%) was applied to aphid-infested cauliflower plants 7 to 10 inches high by sprinkling the material directly over the center of the plant row; each treatment plot was 25 feet long. The material was applied at rates to give 0.23, 0.46, and 0.92 pounds of toxicant per acre. With this method of treatment, much of the granulated material is retained by the leaves or falls into and is held where the leaves are attached to the plant. The results of this test are presented in table 7.

A substantial reduction in the aphid population occurred in all treatments within 5 days after application.

Table 7.—Effect of granulated Bayer 19639 on cabbage aphid populations on cauliflower plants.[a]

Material	Toxicant per Acre (Lb.)	Results per 40 Plants Examined		No. Aphids Before and After Treatment per 4 Tagged Plants[b]	
		July 7			
		Number of		July 2 (Pretreatment)	July 7
		Aphids	Infested Plants		
Bayer 19639	0.92	2	2	350	2
	0.46	37	4	295	2
	0.23	13	7	280	1
Untreated	—	1,587	20	370	754

[a] Granules applied to foilage July 2, 1958.
[b] One heavily infested plant in each replicate was marked and counted at time of application and again 5 days later.

Table 8.—Effect of granulated Bayer 19639 (2.5%) and Thimet (2%) on cabbage aphid populations on cabbage.[a]

Material	Toxicant per Acre (Lb.)	Results per 40 Plants Examined					
		August 1		August 8		August 15	
		Number of		Number of		Number of	
		Aphids	Infested Plants	Aphids	Infested Plants	Aphids	Infested Plants
Bayer 19639	0.76	9	4	26	6	32	11
	.38	22	3	56	9	86	19
	.19	26	11	300	8	118	20
Thimet	.38	3	2	7	3	140	27
	.19	0	0	73	11	107	24
Untreated	—	1,420	36	3,363	34	1,788	36

[a] Granules applied July 29, 1958 to aphid-infested transplants in furrow prior to covering. Transplant furrows were filled after treatment, thus enclosing any granules not retained on small transplants.

Similar results were obtained with 0.72 pound of Bayer 19639 per acre applied with a hand-operated rotary-type duster, the single nozzle being held over the center of the plant row.

Test No. 8—On July 29, 1958, Bayer 19639 (2½%) and Thimet granules were applied by hand to aphid-infested cabbage transplants in the transplant furrow prior to covering. The transplant furrows were covered after the treatment, thus enclosing any granules not held by the plant foliage. Each plot was 25 feet long. The granulated Bayer 19639 was applied at rates of 0.76, 0.38, and 0.19 pound of toxicant per acre and the Thimet at the two lowest rates. The results of this test are given in table 8.

Owing to the small size of the plants and leaves, most of the material fell into the transplant furrow. Both materials proved very effective in giving a quick kill when applied in this manner. The 10-day count revealed young aphids in the center of some plants. This fact indicated that the effective action of the material was diminishing.

Test No. 9.—On July 31, 1958, granulated Bayer 19639 (2½%) and Thimet were applied by hand directly over the foliage of heavily infested cabbage plants 5 to 6 inches tall. Bayer 19639 was applied at 0.79 and 0.38, and Thimet at 0.69 pounds of toxicant per acre. The results of this test appear in table 9.

At the time of application the plants were dry and wilted as well as twisted with aphid damage. On August 5 all treatments had reduced the aphid population by more than 90%. The field was irrigated August 5, and this may have increased the intake of toxicant, in turn accounting for the further decrease in aphid population August 12 in the Bayer 19639 plots, although the effectiveness of

Table 9.—Effect of granulated Bayer 19639 (2.5%) and Thimet (2%) on cabbage aphid populations on cabbage.[a]

Material	Toxicant per Acre (Lb.)	Results per 40 Plants Examined					
		August 5		August 12		August 20	
		Number of		Number of		Number of	
		Aphids	Infested Plants	Aphids	Infested Plants	Aphids	Infested Plants
Bayer 19639	0.79	233	12	99	2	20	1
	0.38	502	10	12	4	15	2
Thimet	0.69	222	6	1,079	8	47	7
Untreated	—	6,220	20	3,994	12	275	4

[a] Materials applied directly onto plant foliage July 31, 1958.

Thimet had decreased on this date. The materials had little or no effect on parasite and predator activity as evidenced by the number of adult hymenopterous parasites as well as syrphid larvae present. The abundance of beneficial insect activity is obvious by the sharp decrease in aphids by August 20.

RESIDUE STUDIES.—Plant samples for residue analysis were taken from four tests in which materials were applied to the soil and one test in which materials were applied to the foliage. Residue samples following soil application were taken from test 3 (table 3), test 4 (table 4), and test 6 (table 6). The rates and methods of application have been described. Two tests were made for residue study only; in the first, the materials were applied to the soil and in the second to the foliage. They are briefly described as follows: On May 10, young cabbage plants were transplanted into furrows in which granulated Bayer 19639 and Thimet had been applied just previously at the rate of 1 pound of toxicant per acre. Irrigation followed immediately after filling the furrows with soil. Each treatment was replicated four times and each replicate was three rows wide and 30 feet long. Samples for analysis were taken 28, 51, 69, and 87 days after treatment. In the other test, granulated Bayer 19639 and Thimet were applied to foliage of young cabbage plants 8 to 10 inches high just prior to heading. Bayer 19639 was applied at 0.38 and 0.19, and Thimet at 0.38 pound per acre. Each treatment was not replicated and was a single row wide and 25 feet long. Four heads were selected from each treatment 21 days after application for residue analysis.

Harvest samples consisted of three to four cabbage heads per treatment, selected at random from each replicate. The sample heads were cut at the base of the stem to include all the leaves on the plant. The heads were quartered, a single quarter of each head entering into the final sample. The composited samples were then run through a bread slicer and quartered down to a 100-gm. subsample. The subsample was homogenized with 100 ml. pH 7.0 water in a Waring Blendor and filtered through three layers of cheesecloth. The average volume of filtrate was 146 ml. Appropriate aliquots of the filtrate were taken and residues determined by the electrometric cholinesterase procedure described by Hensel *et al.* (1954). In this method, human blood plasma is used as the cholinesterase source. Fortification experiments with varying amounts of inhibitor added gave from 76 to 82% recoveries.

The residues were calculated as Thimet oxygen analogue sulfone (*O,O*-diethyl S-(ethylsulfonyl)-methyl phosphorothiolate) for Thimet and as Systox thiol-isomer sulfone (*O,O*-diethyl S-2(ethylsulfonyl)-ethyl phosphorothiolate) for Bayer 19639. These standards were selected in view of previous work by Metcalf *et al.* (1957), who established that both Thimet and Bayer 19639 are ultimately metabolized in plants to the respective phosphorothiolate sulfones.

The residue information is summarized in table 10. It is apparent from the data in table 10 that Thimet residues are somewhat less persistent than Bayer 19639 residues following soil applications. The data are in good agreement with the shorter residual activity of Thimet compared with Bayer 19639 in aphid control.

Table 10.—Residues of granulated Bayer 19639 and Thimet in cabbage following various methods of application to soil and foliage.

Date	Treatment: Material	Treatment: Toxicant per Acre (lb.)	Days After Treatment Sampled	Residues Calculated as Oxygen Analogue Sulfone (P.P.M.)
	Soil Applications (1957)			
February 11[a]	Bayer 19639	1	73	<0.10
	Thimet	1	73	< .02
April 5[b]	Bayer 19639	1	83	< .10
		2	83	.30
	Thimet	1	83	< .02
		2	83	.02
August 7[c]	Bayer 19639[c]			
	(outer leaves)	2	54	.10
	(inner leaves)	2	54	.10
	Thimet[c]			
	(outer leaves)	2	54	< .02
	(inner leaves)	2	54	< .02
May 10[d]	Bayer 19639	1	28	1.54
		1	51	.38
		1	69	.17
		1	87	.13
	Thimet	1	28	.83
		1	51	.03
		1	69	< .02
		1	87	< .02
	Foliage Application (1958)			
August 16[e]	Bayer 19639	0.38	21	<0.10
		.19	21	< .10
	Thimet	.38	21	< .02

[a] Small cabbage plants transplanted into furrows immediately after treatment of the furrows with 2% Bayer 19639 and 2% Thimet granules. See test 3 and table 3 in text.

[b] Young cabbage plants transplanted into furrows which were treated 9 days previously with 2% Bayer 19639 and 2% Thimet granules. See test 4 and table 4 in text.

[c] Young cabbage plants transplanted into furrows immediately after treatment of the furrows with Bayer 19639 and Thimet granules. See test 6 and table 6 in text. In taking plant samples, 10 to 12 of outer (older) leaves were processed as a separate sample.

[d] Young cabbage plants transplanted into furrows immediately after treating the furrows with Bayer 19639 and Thimet granules. See description in section on Residue Studies.

[e] Granulated Bayer 19639 and Thimet applied to foliage of cabbage plants just prior to heading. See description in section on Residue Studies.

DISCUSSION.—Soil applications of granulated Bayer 19639 and Thimet should be placed as near the plant as possible. It appears essential that the plants be in good growing condition and that sufficient soil moisture is present to permit absorption of the material. The absorption of these materials by the plant is gradual and thus prolongs the effective killing action, which in turn increases the residue hazard.

The effectiveness of granulated applications to the foliage depends on the amount of foliage present and its ability to direct the granules toward the center of the plant. Also, that the plants be in good growing condition appears essential, so that there is sufficient moisture at the leaf axils to enable the toxicant to be absorbed. The actual amount of material absorbed into the plant, although sufficient to be effective, is apparently quite small, and thus reduces the residue problem.

The cabbage aphid will attack cabbage at any stage of growth, but it is most damaging prior to head formation. An infestation at this time may not only stunt and distort the plant growth, but may also enable the aphid to penetrate the loosely folded leaves and enter the central portions of the plant. As the plant matures, the aphids become enclosed within the head. The result is an unmarketable crop at harvest time.

The sheltered habits of this insect have made it very difficult to control with contact insecticides, in many in-

stances leading to repeated dust or spray applications. Application schedules of this type, although they may provide the desired control, are not only costly but often subject to interruption by field and weather conditions which limit the type of equipment and application possible. Systemic insecticides, owing to their ability to penetrate the plant and their longer residual action, have been superior to contact poisons in giving control of the cabbage aphid. Previously the use of systemics necessitated spray equipment. Now, however, with the development of granulated systemic insecticides, spraying may be no longer necessary.

Fixed-schedule treatments often preclude the development and eventual utilization of any biotic agents attacking this aphid or other pest species present on this crop. Certainly the placement of these granulated systemic materials in the soil or on the plant foliage has increased their selectivity by minimizing exposure to other than phytophagous species. The idea of applying systemic insecticides in a manner which increases their selectivity is not new. Ripper *et al.* (1951) demonstrated control of certain sucking insects through soil applications of various systemic compounds. The high initial mortality of natural enemies resulting from foliar applications was thus avoided, and the natural enemies were able to continue their attack on the surviving pests. Ahmed (1955) has demonstrated that insect predators feeding on aphids on cabbage sprayed with demeton or schradan suffered a higher mortality than those feeding on aphids killed by a systemic applied to the plant roots.

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Intensive Limited Area Control of Potential Arthropod Disease Vectors[1]

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ABSTRACT

Field studies on intensive limited area control of insects and mites were conducted at Woodbridge, Virginia, during July 1958. The studies were made in order to field test military insect-control equipment and obtain information useful in controlling arthropod disease vectors under emergency conditions, both civil and military.

Natural populations of mites, mosquitoes, and other insects were sampled during a pretreatment period in a control and treatment area. In addition, from 100,000 to 200,000 house flies (*Musca domestica* L.) were released in the treatment area. Intensive daily insecticide applications were made in the treatment area starting July 15, and continued for 10 days. A sharp drop in natural populations took place in the treatment area following the first insecticide application. The same sharp drop did not occur in the control area. During the treatment period mosquito light trap collections showed an average daily reduction of 90%, adult mosquito landing counts 81%, mite population 76%, and general sweep collections 94%. The introduced house flies were difficult to bring under control and more studies along these lines are needed. After insecticiding stopped mosquitoes moved back into the area quickly, light-trap collections went up sharply within 48 hours. The mite and general insect populations showed no signs of a buildup during the week after insecticiding stopped.

Arthropod-borne disease and annoyance are serious problems in peacetime civil disasters. In addition, the possibility exists that arthropod disease vectors capable of transmitting human and other animal or plant diseases will be used in any future war. These same arthropods would be particularly destructive to the military and civil populations following nuclear warfare. Sanitary facilities would have broken down and human resistance to disease would be low at this time.

It is evident that militarily there is a need for the capability to enter an area of limited size and achieve quick knockdown and continued control of arthropods. These studies were undertaken to obtain needed information on this type of intensive limited area control. A knowledge of this method of control, particularly as it relates to the available insecticide dispersal equipment, is essential for developing the most effective prevention and control measures.

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METHODS AND MATERIALS.—This study was conducted at a military installation at Woodbridge, Virginia, from July 7 through August 1, 1958. The site was on fairly level ground and was covered with a dense growth of blackberry bushes and grass; it was surrounded by wooded and swampy areas. It was divided into a treatment area of 40 acres and a control area of 20 acres. The two areas were similar in physical characteristics and were separated by approximately a quarter of a mile.

The period July 7 through 13, 1958, was utilized to determine the natural insect and mite populations in the control and treatment areas prior to the application of insecticides. Surveys were conducted to determine the indices of mites, mosquitoes, and general insect populations.

Mite populations were determined by using eleven 2×6 inch black plastic plates, ¼ inch thick. Each day the plates were placed on the ground under blackberry bushes and allowed to remain 10 minutes. The mites on the plates were then counted and removed with a small brush. The 11 plates were placed 3 times daily in different locations in the treatment and 3 times in the control areas. This resulted in a total of 33 plate counts in each area daily.

The mosquito index was determined by mosquito light traps and biting collections. Mosquito light traps were operated from 6 a.m. to 6 p.m. daily, two in the control and four in the treatment area. Electric power was available from a powerline only in the control area; a 10-kw. generator was used as a power source in the treatment area. Mosquito biting collections of 5-minute duration were made twice weekly beginning approximately at 9 p.m. These collections were taken only in the treatment area, as the personnel available was limited and did not permit collections to be made concomitantly in the control area.

General insect populations were determined by using an insect sweep net. The net was swept over the vegetation in each area daily and the arthropods were transferred to a holding jar and chloroformed. The specimens collected were counted and identified to order. Initially 100 sweeps were taken daily in each area. However, the number obtained was so large that the number of sweeps was ultimately reduced to 10.

Laboratory-reared house flies (*Musca domestica* L.) were released in the test area in order to obtain information on the effectiveness of intensive limited area control against an introduced insect population. From 100,000 to 200,000 house-fly larvae and pupae with their rearing media were dumped in the center of the treatment area on July 14, 1958. These flies were provided by the Army Environmental Health Laboratories, Edgewood, Maryland. Fly populations were determined with Scudder fly grids. The grids were placed on the ground adjacent to the breeding media and permitted to remain for 1 minute, then flies observed on the grid were counted. Ten 1-minute observations were made daily.

Test models of a trailer-mounted sprayer and duster, and a skid-mounted fog machine, currently being developed by the Engineer Research and Development Laboratories, were used for dispersing insecticides. From July 15 through 25, insecticides were applied daily in the 40-acre treatment area with these machines. During this 10-day period, 760 gallons of 10% DDT spray and fog, and 220 gallons of 2.7% malathion fog were dispersed in the treatment area. This treatment resulted in a dosage of 15.2 pounds actual DDT and 1.04 pounds actual malathion per acre. In addition, on July 19 an application of 1,000 pounds of 10% DDT dust was made at the rate of 25 pounds, 2.5 pounds actual DDT per acre. The total amount of actual insecticide applied during the 10-day treatment period was 18.74 pounds per acre. Sampling of the arthropod populations identical to sampling procedure previously described was continued during the entire insecticiding period.

RESULTS.—Mosquito light traps were operated for 22 nights, 4 traps in the treatment area and 2 traps in the control area. The mosquito populations were sampled for 6 nights during the pretreatment period, 11 nights during treatment, and 5 nights starting 24 hours after application stopped. A total of 3,302 mosquitoes from the treatment area and 4,717 from the control area representing 12 species was identified.

Many of the specimens were identifiable only to tribe or genus. Of the identifiable species *Aedes vexans* (Meigen) made up 75% and *Culex salinarius* Coquillett 22.6%. The remaining 10 species, *Aedes sollicitans* (Walker), *Anopheles punctipennis* (Say), *Anopheles crucians* Wiedemann, *Culex restuans* Theobold, *Culex territans* (Walker), *Psorophora confinnis* (Lynch-Arribalzaga), *Psorophora ciliata* (Fabricius), *Psorophora discolor* (Coquillett), *Psorophora cyanescens* (Coquillett), and *Uranotaenia sapphirina* (Osten-Sacken) comprised only 2.4% of the collections.

The four mosquito light traps in the treatment area averaged 88 mosquitoes per trap night during the pretreatment period. During the 11 nights that insecticides were being used the average number of mosquitoes per trap night dropped to nine, a 90% reduction. The reduction in mosquito populations was very sharp after the first application of insecticide. On the night of July 14, the four traps in the treatment area caught an average of 62 mosquitoes per trap. After the first applications of insecticide on July 15, the same four traps for the night of July 15, averaged only six mosquitoes per trap. The mosquito populations were not eradicated from the treatment area but were reduced to very low levels and held there until treatment stopped. After insecticide applications stopped the populations were sampled for 5 nights, and average number of mosquitoes collected per trap night climbed to 40.

Insecticides were not used in the control area, and during the sampling mosquito populations were higher in the control than in the treatment area. On the night of July 14, the two traps in the control area averaged 105 mosquitoes. The collections for the night of July 15, did not drop acutely as in the treatment area, but averaged 112 mosquitoes. This increase over the previous night was seven.

Landing counts of adult mosquitoes were made eight times in the treatment area, twice in the pretreatment period, four times during treatment, and twice after insecticiding stopped. In the two pretreatment counts made on July 7 and 9, the average number of mosquitoes collected was 27. During treatment, counts made on July 16, 17, 21, and 24 averaged 3, and 81% reduction.

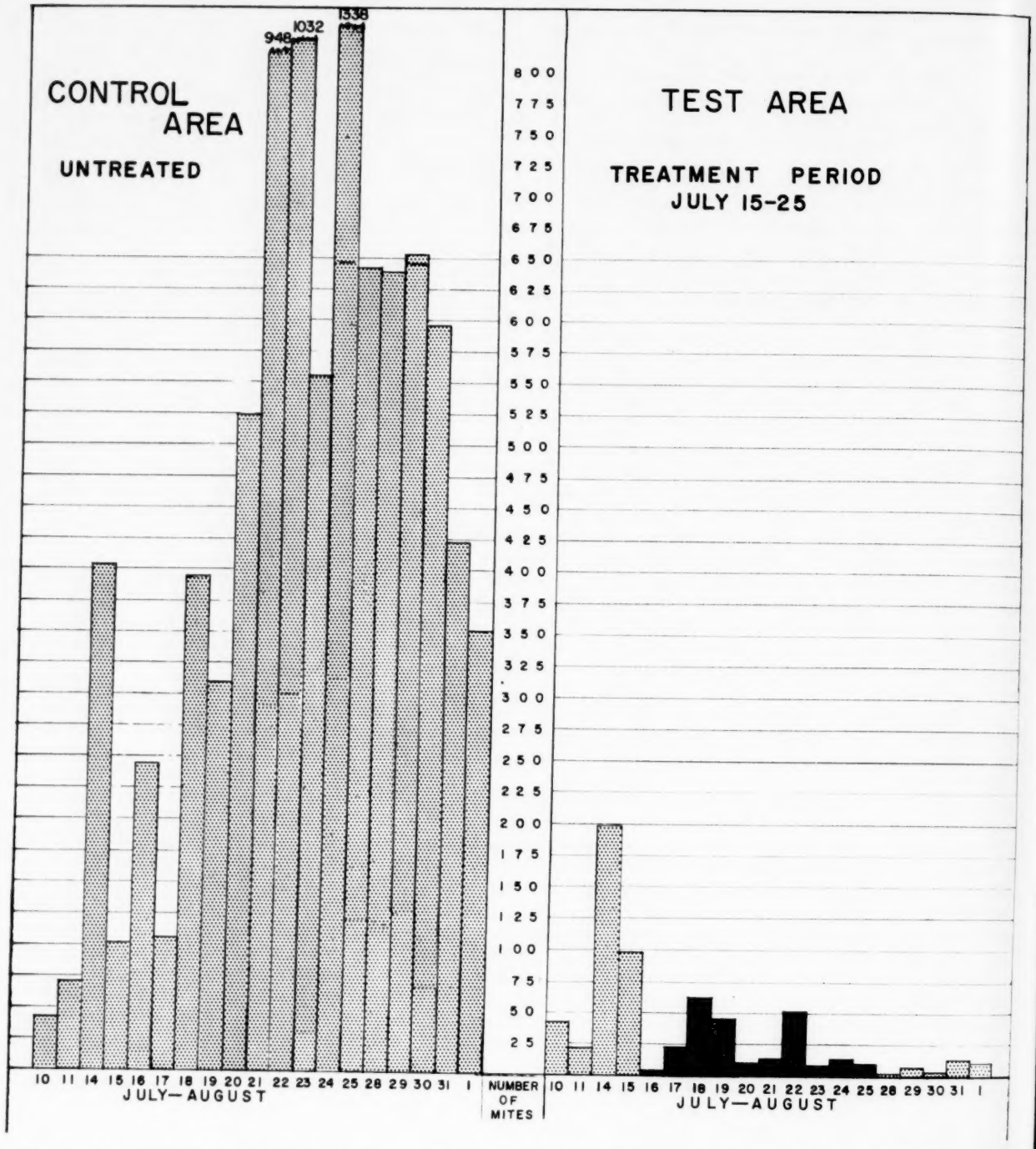

FIG. 1.—A comparison of mite collections from treated and untreated areas, Woodbridge, Virginia, July 17 to August 1, 1958.

After insecticiding stopped, landing counts made on July 28 and 29 averaged 15.

Mite populations were determined for 19 days, 4 days during the pretreatment period, 10 days during treatment, and 5 days starting 48 hours after insecticiding stopped. A total of 685 mites from the treatment area and 9,304 from the control area were collected. The mites were not identified to species but the total number collected was considered significant.

Figure 1 shows a daily comparison of the mite populations from the treatment and control areas. In the treatment area the mite populations dropped sharply with the first application of 10% DDT spray on July 15, and the populations were kept low by repeated applications of 10% DDT spray on July 18, 10% DDT dust on July 19 and 20, and 10% DDT spray on July 23. After the insecticide applications stopped the mite populations were sampled for 5 days and remained at a very low level.

The general insect populations were sampled with sweep nets for 20 days, 5 days during the pretreatment

period, 10 days during treatment, and 5 days starting 48 hours after insecticiding stopped. A total of 768 insects from the treatment area and 1,374 from the control area were identified to order. Nine orders were identified from the sweep collections: Orthoptera, Hemiptera, Homoptera, Diptera, Coleoptera, Hymenoptera, Lepidoptera, Neuroptera, and Thysanoptera.

In the treatment area the general insect populations were sampled 5 days before insecticides were applied. The average number of insects collected daily during this 5-day pretreatment period was 135. During the 10-day treatment period, the average number of insects collected daily dropped to 7, a 94% reduction. The reduction in general insect populations was very sharp after the first application of insecticides. Three hundred and fifteen insects were collected in the treatment area on July 14, insecticides were applied July 15, and only 2 insects were taken in 10 sweeps on July 16. After the insecticide applications stopped the general insect population was sampled for 5 days and remained at a very low level.

In the control area the general insect populations sampled during the 5-day pretreatment period averaged 73. During the 10-day period, equivalent to that in which insecticides were used in the treatment area, the general insect populations went up slightly, averaging 78 daily.

From 100,000 to 200,000 house flies were released in the treatment area on July 14, 1958. The rearing media containing larvae and pupae were dumped in the treatment area at 2:00 p.m. Ten 1-minute counts using Scudder fly grids were made at 4:00 p.m., and a total of 46 flies was observed. The following day, July 15, three counts were made before insecticiding, 32 flies were observed at 7:00 a.m., 47 flies at 10:30 a.m., and 26 flies at 3:15 p.m. The treatment area was then sprayed and fogged with DDT and malathion.

Following the application of insecticides a daily count was made near the center of the treatment area. On July 16, a total of 58 flies was observed on the grids. This was the peak of fly populations and was due in part to continued emergence. The count dropped to 14 on July 17, and stayed at 14 or less for the next 5 days. The count dropped to zero on July 23, and remained at that point until the test ended.

DISCUSSION.—Under emergency conditions, both military and civil, results must often be achieved quickly in controlling vector borne diseases such as dysentery, encephalitis, murine typhus, and others. In addition to those diseases which now occur in our country, other diseases or those presenting only a small problem under normal conditions may be introduced by enemy action. These could be spread by natural or introduced vectors. Studies were made at Woodbridge, Virginia, to determine if presently available equipment and insecticides could be utilized for intensive limited area control of insects and mites. If the time comes when results must be achieved quickly, then experience gained in these studies would be applicable at any military installation or civil disaster area.

One application of 10% DDT spray and 2.7% malathion fog brought about a sharp reduction in all of the natural insect and mite populations within 24 hours. During the 10 days that repeated applications of these insecticides were made in the treatment area the natural populations were held at low levels. The sudden sharp drop observed in the treatment area did not occur in the control area where populations actually went up. After insecticiding stopped the mosquitoes moved back into the area quickly, light-trap and biting collections went up. The sweep collections of general insect populations and mite collections did not build up when insecticiding stopped, but stayed at low levels during the posttreatment counts.

The house flies that were introduced into the treatment area were more difficult to control than the natural insect populations. Following the first application of spray fog on July 15, the fly populations went up reaching a peak on July 16. The following day the fly populations dropped but persisted in the treatment area until July 22.

The fact that it was difficult to control an introduced population is important. It is possible for entomologists to rear great numbers of insects that are resistant to insecticides in the laboratory. If large numbers of insecticide-resistant arthropods were infected with disease and released in a limited area, quick detection and control, though difficult, would be essential. Such enemy action is possible, and considerable additional research on detection and control is needed.

The Trend of DDT and Parathion Residues on Apples Grown in Central Washington[1]

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ABSTRACT

A study was carried out in 1958 to determine the average amounts of DDT and parathion residue on apples in central Washington and to compare these residues with those found in past years when fewer and earlier sprays and lower dosages were used. The DDT residue levels ranged from 0.3 to 12.4 p.p.m. with a mean of 3.0 p.p.m. for all samples, both washed and unwashed. For each variety and for all samples considered together, the unwashed apples had a higher DDT residue than the washed samples. Only nine of the unwashed samples (15.2%) and 1 washed sample (1.6%) had residues above the 7 p.p.m. tolerance level. Parathion residue ranged from 0.01 to 0.32 p.p.m. with a mean of 0.10 p.p.m. The residue values for both DDT and parathion reported here are somewhat higher than those reported for earlier years by various workers. With an increase in number of cover sprays applied, a corresponding increase in DDT residue was observed. Washing was found to lower the DDT residue level, but little difference between washing with and without brushes was apparent.

[1] From the Communicable Disease Center, Bureau of State Services, Public Health Service, U. S. Department of Health, Education and Welfare, Wenatchee, Washington. Accepted for publication April 24, 1959.

Since the first general use of DDT and parathion for insect control on tree fruits in central Washington was made during 1947–48 the amount of these insecticides used each season to maintain adequate control has gradually increased. Although there has been a small increase in the dosage rate of DDT during this period, the major changes contributing to the residue problem have been the application of a greater number of cover sprays containing DDT during the growing season, and the fact that the last cover spray is often applied closer to harvest. The dosage rate and number of parathion sprays have also increased.

A large proportion of orchardists applied DDT sprays to their apples during 1947, and by 1948 practically all the apple orchards were sprayed with DDT. Parathion became available to orchardists during midseason of 1947, and by 1948 it was being widely used as a supplemental material in the spray program. In most instances only one or two DDT cover sprays were necessary for codling moth (*Carpocapsa pomonella* (L.)) control. Only a few orchards required more than three covers. During this period most orchardists used the dosages recommended by the State College of Washington (Anonymous 1948), *i.e.*, 1 pound of actual DDT per 100 gallons of water in the first cover spray, 0.5 to 1 pound in the second and third covers, the last cover to be applied not later than 30 days prior to harvest. In the second and third cover sprays a high percentage of orchardists used the 0.5-pound rather than the 1-pound DDT dosage. Parathion was recommended as a supplemental material where aphids and mites were a problem, at dosages of from 1 to 2 ounces of actual parathion per 100 gallons of water, with the spray not to be applied later than 30 days prior to harvest. During 1947 and 1948, parathion generally was used in only one or two cover sprays.

Westlake & Fahey (1950) analyzed samples of apples from the Yakima Valley area of Washington that were sprayed during the 1948 season. They found from 1.0 to 1.7 p.p.m. of DDT on unwashed Delicious that had received one and two cover sprays, and 2.7 to 3.3 p.p.m. on unwashed Winesap apples that had received three cover sprays. Westlake and Fahey also reported from 0.02 to 0.07 p.p.m. parathion on these apples.

A check of the 1949 spray programs of 100 orchardists revealed that 25% applied one DDT cover spray, 47% applied two, 24% applied three, and 4% applied four DDT cover sprays. A high percentage used 1 pound of actual DDT per 100 gallons of water in all cover sprays. Only 15% of these 100 orchardists had added parathion to their cover spray program, and most of the parathion used was applied in the second and/or third cover.

During the 1949 season, chemists at the State College of Washington Tree Fruit Experiment Station, Wenatchee, Washington (Walker 1950), analyzed many unwashed apples for DDT and parathion residue. They found an average of 2.0 p.p.m. of DDT on Delicious, 2.7 on Golden Delicious, 2.5 on Winesap, and 1.7 on Jonathan. The average amount of parathion found was less than 0.03 p.p.m. for all varieties.

By 1951 the picture as far as number of DDT sprays applied per season had not changed much, but in checking the programs of 100 orchardists that year it was noted that more than 50% used dosages, in at least one cover spray, which were less than 1 pound of actual DDT per 100 gallons of water. The use of parathion, however, was on the increase. Of the 100 orchardists, 13% used parathion in one cover spray, 72% used it in two covers, and 15% used it in three cover sprays. Recommendations by the State College of Washington (Anonymous 1951) at that time were: 1 pound of actual DDT per 100 gallons of water in the first cover spray, 0.5 to 1 pound in the second, and 1 pound in the third. Recommendations for parathion suggested dosages from 1.3 to 4 ounces of actual parathion per 100 gallons of water in from one to two cover sprays.

At the end of the 1951 growing season this laboratory collected washed fruit samples for DDT residue determinations from numerous commercial packing warehouses in central Washington. Out of 90 samples, 17 had received one cover spray; 53, two; 17, three; 2, four; and 1, five. The average amount of DDT residue found on the surface of the fruit was 1.2 p.p.m. for Delicious, 2.0 for Golden Delicious, 2.8 for Winesap, and 1.2 for Jonathan.

This laboratory also analyzed fruits grown in this area during the 1954 season and found an average residue of 1.6 p.p.m. of DDT on Winesap. No difference was found in residue level between washed and unwashed samples.

The results of studies of DDT residues on apples grown in central Washington for 1948, 1949, 1951, and 1954 are summarized in table 1.

By 1957–58 more orchardists were finding it necessary to apply four cover sprays and, especially during 1958, a few growers applied five sprays containing DDT. Many of these sprays were applied near to or even beyond the 30-day deadline prior to harvest. By 1958 most orchardists were applying all sprays of DDT at 1 pound actual per 100 gallons of water, and many were also using parathion in practically every cover spray at the rate of 0.25 pound actual per 100 gallons of water. Recommendations allowed application of parathion within 14 days of harvest.

In a study carried out during 1954–56 by Hayes and co-workers (1958), it was shown that no progression in the storage of DDT in the general population of the United States has occurred since this storage was first measured in 1950. This finding indicates that the DDT content of the total diet was not significantly increased during this period.

The purpose of the present study was to determine the average amount of DDT and parathion residues on apples sprayed during the 1958 season in central Washington, and to compare these residues with those determined in past years when fewer sprays, earlier sprays, and lower dosages were used. A study was also made of the effect on residue of a few factors operative during the growth season. The factors studied included formulation, dosage, number of sprays applied, and date of last application.

In addition to the factors listed above, apple packers and processors, as well as growers, have exhibited considerable interest regarding the efficiency of warehouse washing procedures in removing residue. Most apple packing warehouses in central Washington have at least one machine that can be equipped with brushes for the purpose of more thoroughly cleaning the fruit. The water-washing process, whether with or without brushes, is effective in removing visible residue. However, the effectiveness of these procedures in removing oil-soluble residues such as DDT has needed further exploration.

Table 1.—DDT residues (p.p.m.) found at harvest on washed and unwashed apples grown in central Washington during the crop years 1947–58, as reported by various investigators.

Variety	Value	Investigator and Crop Year						
		Westlake & Fahey 1948	Walker 1949	U. S. Public Health Service				
				1951	1954		1958	
		Unwashed	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed
Delicious[a]	No. of samples	5	531	55	—	—	38	44
	Range	1.0–1.7	0.0–7.9	0.2–4.0	—	—	0.3–12.3	0.3–7.0
	Mean±S.E.[b]	1.3±0.1	2.0	1.2±0.1	—	—	3.4± 0.6	2.3±0.1
Golden Delicious	No. of samples	—	36	8	—	—	10	7
	Range	—	0.0–5.5	0.2–4.6	—	—	0.5–6.3	0.3–3.0
	Mean±S.E.	—	2.7	2.0±0.4	—	—	3.1± 0.7	1.5±0.5
Winesap	No. of samples	2	294	32	14	21	8	8
	Range	2.7–3.3	0.0–11.0	0.1–5.1	1.0–3.0	1.0–4.0	0.4–12.4	0.5–8.2
	Mean±S.E.	3.0±0.3	2.5	2.8±0.2	1.6±0.1	1.6±0.2	5.4± 1.2	3.9±0.8
Jonathan	No. of samples	—	129	2	—	—	3	5
	Range	—	0.0–5.3	1.0–1.3	—	—	1.6–7.6	0.7–3.7
	Mean±S.E.	—	1.7	1.2±0.1	—	—	5.1± 1.8	1.5±0.6

[a] Red and Standard varieties.
[b] Standard error of the mean.

Robinson (1947) reported that washing apples in water immediately after harvest removed about 30% of the residual DDT. Walker (1948) reported that none of the washing treatments he tested effectively reduced high DDT residues. Walker (1949) further noted that when the original DDT residues on apples exceeded 10 p.p.m., the standard washing practices in north central Washington were not sufficient to consistently reduce the residual deposit below the 7 p.p.m. tolerance level.

For these reasons, evaluation of the efficiency of presently used warehouse washing procedures was included in this study.

METHODS AND MATERIALS.—Apple samples for the 1958 residue study were collected from commercial packing warehouses. Most of the Delicious and Jonathan samples were taken within 2 weeks after harvest and none was in storage longer than 8 weeks. Although a few Golden Delicious and Winesap samples were taken soon after harvest, most of these two varieties were taken after they had been in storage 4 to 8 weeks. Unwashed samples were taken just prior to their entrance into the washer, and washed samples were taken from the grader bins after they had been water washed, sorted, and sized by weight preparatory to packing for shipment to market. The apples ranged from sizes 125 to 138 (2 14/16 to 2 13/16 inches in diameter). Sufficient firm fruit to completely fill a 1-gallon, wide-mouthed glass jar was washed with 750 ml. of hexane (Skellysolve B) for 15 minutes in an end-over-end type tumbling machine, and aliquots of the hexane extract were taken for analysis. Determinations of DDT residues were carried out by the method of Schechter *et al.* (1945), as modified by Mattson *et al.* (1953). Parathion analyses were made by the method of Averell & Norris (1948).

RESULTS.—*DDT Residues.*—A total of 123 samples of apples was analyzed for DDT residue. These samples had received from one to five DDT cover sprays. DDT found on the fruit ranged from 0.3 to 12.4 p.p.m., with an average of 3.0 p.p.m. on all samples, both washed and unwashed. Unwashed samples averaged 3.7 p.p.m. and washed samples averaged 2.4 p.p.m.

Table 1 shows the amount of DDT found on each of the four varieties of apples, unwashed and washed. As has been reported by other workers, slightly higher residues were found on Winesap than on the other varieties. For each variety and for all samples considered together, the unwashed apples had a significantly higher residue than the washed samples. Only nine unwashed (15.2%) and one washed sample (1.6%) had residues above the 7 p.p.m. tolerance level. As indicated in table 1, the DDT residue values for the 1958 crop are somewhat higher than in previous years for which similar studies are available.

Table 2 shows the relationship of the number of cover sprays applied during the season to the amount of DDT residue. The data presented show that with an increase in the number of cover sprays applied, a corresponding increase in the amount of residue is found on the fruit. It is interesting to note that, of the 12 samples that received five cover sprays, five (42%) exceeded the tolerance level. Even the mean value for this group of samples

Table 2.—Relation of number of cover sprays applied to apples to the amount of DDT residue found at harvest.

No. of Cover Sprays	Average Interval Between Last Spray and Harvest (Days)	No. of Samples	Residue (P.P.M.)	
			Range	Mean ±S.E.[a]
1	60.0	2	0.9 to 0.9	0.9±0.0
2	51.1	14	.3 to 3.7	1.5±0.3
3	38.9	61	.3 to 8.2	2.6±0.3
4	23.9	34	1.1 to 7.6	3.3±0.3
5	17.2	12	1.1 to 12.4	6.4±1.0

[a] Standard error of the mean.

Table 3.—Effect of warehouse washing procedures on DDT residue on apples.[a]

Residue Level	DDT Residue (p.p.m.) After Indicated Treatment		
	Unwashed	Washed, No Brushes	Washed, Brushes
High			
Range	9.0–12.3	2.7–3.1	3.4–4.9
Mean±S.E.[b]	10.6± 1.0	2.8±0.1	4.0±0.5
Medium			
Range	5.4– 6.2	2.0–3.7	2.6–4.5
Mean±S.E.	5.9± 0.3	2.7±0.5	3.8±0.6
Low			
Range	2.0– 2.6	1.1–1.3	1.2–1.4
Mean±S.E.	2.4± 0.2	1.2±0.1	1.3±0.1
Mean			
Range	2.0–12.3	1.1–3.7	1.2–4.9
Mean±S.E.	6.3± 1.0	2.2±0.3	2.9±0.5

[a] Each mean represents the average for three replicate samples.
[b] Standard error of the mean.

(6.4±1.0 p.p.m.) approaches the 7 p.p.m. figure. These results suggest that the application of more than five cover sprays may give residue values of such magnitude that virtually all samples may exceed the tolerance level.

The mean time intervals between last spray and harvest for samples having different numbers of cover sprays are also shown in table 2. It is apparent that a negative correlation exists between interval from last spray to harvest and DDT residue level. However, the number of cover sprays and time interval between last spray and harvest are also interrelated, so that, in general, as the number of cover sprays is increased, the interval between last spray and harvest will become shorter. In order to separate the relative magnitude of the effects of these two interrelated factors on residue level, statistical analysis involving determination of the partial correlation coefficients for the effect of number of cover sprays and of interval between last spray and harvest on residue level was carried out by the method described by Snedecor (1956). The effects of both number of cover sprays and of interval between last spray and harvest on residue level was found to be significant at greater than the 99% level. The correlation coefficient for residue level with number of sprays (+0.475) was somewhat greater than that with interval between last spray and harvest (−0.403).

An additional factor that might be expected to influence the residue level at harvest is the formulation used, particularly the presence or absence of a spreader or sticker-type adjuvant in the spray mix. Little information was obtained on this point, however, since only four of the apple samples tested had been sprayed with formulations containing a spreader or sticker. The material used on the test samples was X-77, a combined spreader-activator, manufactured by Colloidal Products Corporation, Sausalito, California. The principal functioning agents in this material included alkyl arylpolyoxyethylene glycol, isopropanol, and free fatty acids. Two to four ounces were used per 100 gallons of spray mixture. These four samples (two Delicious and two Winesap) had residues before washing of from 1.3 to 5.8 p.p.m. with a mean of 3.2 p.p.m.

Effect of Washing Equipment.—In order to define more clearly the effectiveness of various types of washing equipment in DDT residue removal, an experiment was conducted to compare the residues on unwashed fruit, on fruit that had been water-washed in a machine without brushes, and on fruit washed in a machine equipped with brushes. Fruit samples from lots where the approximate residue level was known were used to set up a replicated experiment, including samples of relatively high, medium, and low residues, respectively. Amounts of DDT were determined on three replicate samples of apples from each of the three sources, including samples unwashed, washed without brushes, and washed with brushes. The whole sampling procedure was carried out in a commercial packing warehouse and typical commercial washing machines were used. Results are shown in table 3.

On the basis of the data presented, it appears that the washing process removes significant amounts of DDT, especially where higher residues are present before washing. It will be noted that DDT residue on samples containing as high as 12.3 p.p.m. was reduced to levels well below the 7 p.p.m. tolerance when the fruit was run through the washer. It should be emphasized, however, that not all samples having prewash residues in this range will be converted to a sub-tolerance level by washing. One sample (from table 1), for example, was reduced only from 12.4 p.p.m. to 8.2 p.p.m. The 37 other samples that were analyzed both before and after washing showed an average DDT residue of 3.2 p.p.m. before washing and 1.5 p.p.m. after washing.

It is of interest to note that, in the replicated washing experiment, the machine without brushes appeared to be more efficient in removing residue than the machine equipped with brushes. One might speculate that, over a period of time during the early part of the packing season, the brushes would accumulate a deposit of DDT. Once DDT is on the brushes, their action might no longer be to remove DDT from all the fruit, but rather to remove some DDT from fruits with extra heavy deposits and to add small amounts of DDT to fruits having little or no DDT on their surface prior to entering the washer.

Parathion Residues.—A total of 24 unwashed apple samples was analyzed for parathion residue. The varieties sampled included Delicious, Jonathan, and Winesap, which had received parathion in from one to five cover sprays. The amount of parathion found on the fruit ranged from 0.01 to 0.32 p.p.m. with an average of 0.10 p.p.m. The amount found on Delicious averaged 0.09 p.p.m.; on Jonathan, 0.08 p.p.m.; and on Winesap, 0.24 p.p.m. These values are somewhat higher than the 0.02 to 0.07 p.p.m. found by Westlake & Fahey (1950) on 1948 fruit and much higher than the <0.03 p.p.m. average reported by Washington State College Tree Fruit Experiment Station chemists for 1949 fruit (Walker 1950).

No correlation was found between the amount of parathion residue at harvest and the number of parathion sprays applied. The average residue for samples that had received three sprays was 0.11 p.p.m. and for samples that had been sprayed four times was 0.10 p.p.m.

There was a definite correlation between the amount of parathion residue and closeness of spray to harvest. At harvest time on September 15 no parathion residue could be found on apples to which the last parathion spray was applied during May. Where the last spray was applied

during June or July, an average of 0.02 p.p.m. was found. The average amount of parathion found on apples sprayed during August and September was 0.02 p.p.m. for those sprayed 25 to 27 days prior to harvest, 0.12 p.p.m. for those sprayed 18 to 21 days before harvest, and 0.12 p.p.m. for those sprayed only 10 to 15 days before harvest.

All the parathion residue values for apples from the 1958 crop were well below the 1 p.p.m. tolerance level.

DISCUSSION.—Although residues of both DDT and parathion on apples sprayed during the 1958 season in central Washington were somewhat higher than those found in similar studies carried out between 1948 and 1954, the increase in amount of residue resulting from increased dosage and additional, later cover sprays has not been great. In almost all instances, the residues have been below the tolerance level. The small, definite increase noted in residue level for both DDT and parathion over the 10-year period covered, however, emphasizes the need for giving close attention to spray programs in the future. As insect resistance or other factors force more intensive application of DDT and parathion, additional surveys of the effect on residue levels should be carried out.

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Some Aphid Vector-Plant Virus Relationships of the Feathery Mottle Virus of Sweet Potato[1]

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ABSTRACT

A study of the insect vector-plant virus relationships was undertaken with the severe isolate of the feathery mottle virus of sweet potato and the green peach aphid (*Myzus persicae* (Sulz.). The acquisition threshold period was found to be between 0 and 5 seconds. The inoculation threshold period was in the range of 5 to 10 seconds. Preacquisition starvation experiments revealed that maximum transmission efficiency of the virus could be obtained after 5 minutes of starving. Postacquisition starvation results indicated that as starving time increased, the efficiency for transmission decreased. Results of serial transmission trials indicated that 38 out of 50 aphids, starved and given short acquisition feeding periods, were infective; and these insects infected 115 of the 500 plants tested. Analysis of the data, using the binomial theorem, supported the hypothesis that individual aphids were equally charged with virus. When results of artificial and natural interrupted acquisition feedings were compared, no significant difference in transmission efficiency was found. The green peach aphid, the celery aphid (*Aphis apii* Theob.), and the melon aphid (*Aphis gossypii* Glover) were found to be vectors of the virus whereas six other species of aphids tested failed to transmit it.

Although many investigations have been undertaken with reference to mechanical transmission of some of the virus diseases of sweet potato, little extensive research has been conducted with the insect vector-plant virus relationships. Because the feathery mottle virus was found in California and was aphid transmissible (Webb & Larson 1954), it was thought desirable to investigate the relationships of aphid vectors, particularly the green peach aphid, *Myzus persicae* (Sulzer), and the feathery mottle virus. The results may be useful in differentiating various sweetpotato viruses as well as adding information on insect vectors and their relationships with plant virus diseases. Correlations between aphids and whiteflies as vectors were not considered in this study.

The symptoms associated with this virus in sweetpotato were first described by Doolittle & Harter (1945).

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Stubbs & McLean (1958) described symptoms on Jersey variety sweetpotato and on the Brazilian morning glory, *Ipomoea setosa* Ker.

Aphid transmission of the feathery mottle virus was shown by Webb & Larson (1954). Groups of green peach aphids were allowed to feed on diseased sweet potatoes and then transferred to healthy sweet potatoes. Stubbs & McLean (1958) reported studies on two isolates of the feathery mottle virus, designated as severe and mild, which appeared to be nonpersistent and aphid-borne. In host range studies using the green peach aphid as the vector, only some plants in the family *Convolvulaceae* could be experimentally infected.

MATERIALS AND METHODS.—The original feathery mottle virus source plant, a Jersey variety sweet potato, was collected from a field in San Joaquin County, California. Identification of the disease in sweet potato was based on the symptoms described by Doolittle & Harter (1945). The Brazilian morning glory, *Ipomoea setosa* Ker. was used as the test plant unless otherwise noted because of its high degree of susceptibility and striking symptom expression. In all experiments, the test plants used were selected at random from a uniform population. Infected *I. setosa* plants were used as virus sources for most of the experiments with exceptions noted in the text. The green peach aphid was the vector unless otherwise specified. Green peach aphid colonies were maintained on *Brassica juncea* Coss., while plants on which other aphid species were reared are noted in the text. All aphids used were apterous and selected at random from the source colony.

Aphids were transferred with a camel's-hair brush, and most feedings were observed through a 10× hand lens and timed with a stop watch. Usually the aphids were left on the test plants for 1 hour excepting variations as stated in the text. Test plants were fumigated with nicotine sulfate before being placed in the greenhouse for incubation. The plants in the greenhouse were sprayed with nicotine sulfate once or more per week to prevent any buildup of aphid populations that might result in accidental greenhouse spread.

More detailed descriptions of methods and specific techniques are given in connection with individual experiments.

ACQUISITION THRESHOLD PERIOD.—Early data on nonpersistent viruses indicated that the acquisition threshold period was in the 2- to 5-minute range (Doolittle & Walker 1928, Hoggan 1933, Watson 1936). Later data, where the acquisition feeding time was reduced to seconds, the acquisition threshold period of typical nonpersistent viruses was found to be in a 5 to 30-second range (Sylvester 1949, 1955, Bradley 1952).

Experiments were conducted to ascertain the threshold period for the acquisition of the severe isolate of the feathery mottle virus by green peach aphids. The virus source plant used was chosen from *I. setosa* plants that had previously been infected and this source plant was used exclusively for all trials. The acquisition feeding times were: 5, 10, 15, 20, 25, 30, and 35 seconds. The order of use of the acquisition feeding intervals for each replication was chosen at random with the use of a table of random numbers (Snedecor 1956).

Approximately 75 aphids were collected with an aspirator and starved for 1 hour. Single apterous green peach aphids were used for each test and a series of five test plants per replicate were used for each time period with a total of nine replications. In addition, 10 healthy *I. setosa* check plants were used per replication. Following feeding on the source plant, the aphids were transferred to the test plants for 1 hour.

Table 1.—Results of trials conducted to determine the acquisition and inoculation threshold periods for transmission of the severe isolate of the feathery mottle virus by single apterous green peach aphids.[a]

FEEDING TIME (SECONDS)	ACQUISITION	INOCULATION
5	3/45	0/45
10	13/45	2/45
15	23/45	11/45
20	17/45	17/45
25	10/45	19/45
30	16/45	20/45
35	18/45	—
Check	0/90	0/90

[a] A series of five test plants was used per time period per replication with a total of nine replications.

The results of this experiment are presented in table 1. Analysis indicated that from the 10-second feeding to the 35-second feeding, the number of plants infected remained uniform ($\chi^2=9.06$, df = 5, p = ≈0.10). When the 5-second feeding results were introduced, heterogeneity occurred ($\chi^2=24.9$, df = 6, p = <0.001), indicating that the lower efficiency for transmission at this time period was probably not due to chance. The replication totals were tested for homogeneity ($\chi^2=4.85$, df = 8, p = ≈0.80) and the evidence for differences among replications was not significant. Since transmission did occur at the 5-second feeding time, the acquisition threshold period therefore falls somewhere in the range of 0 to 5 seconds.

As was stated earlier, all feeding times were selected at random but a question arose as to whether or not the difference in time of starvation was equally distributed among the treatments. Analysis of variance revealed that it was not ($F=8.17$, $F_{.01}=3.20$). Despite the variability in starvation, no systematic effect on the number of positive infections seems to appear. This fact may be noted with reference to table 2 by comparing the mean starvation times with the corresponding number of infections for each time period tested. It will be observed from this table that although there is wide variation in the number of infected plants with the 5-second feeding and the 35-second feeding, the mean starvation times are quite similar. In addition, the 15-second period shows a higher number of infections than those of the 10- and 20-second periods, although the mean starvation times are not widely varied.

Table 2.—Mean starvation times and the total number of infections produced for each time period tested in relation to tests conducted to determine the acquisition threshold period of the severe isolate of the feathery mottle virus.

Feeding time (seconds)	5	10	15	20	25	30	35
Mean starvation time (minutes)	139.1	123.1	121.7	126.4	142.6	145.1	135.7
No. infected / No. tested	3/45	13/45	23/45	17/45	10/45	16/45	18/45

INOCULATION THRESHOLD PERIOD.—Inoculation threshold period investigations have provided data that indicate several nonpersistent aphid-borne viruses can be inoculated within a 5- to 20-second range (Bradley 1952, Sylvester 1956).

Tests were designed using 1 aphid per plant for each inoculation, a series of 5 test plants per time period for each replication with a total of 9 replications, and 10 check plants for each replication tested. Time periods, in seconds, used for the test feeding were: 5, 10, 15, 20, 25, and 30. The virus source plant used was an infected *I. setosa* obtained from the previous experiment and aphids were collected as described in the acquisition threshold period experiment. Following a 1-hour starvation, single apterous green peach aphids were allowed to feed for 15 seconds on the source plant, artificially interrupted, and transferred to the test plants to feed for the indicated time, after which they were again artificially interrupted and removed. The order of the test feeding intervals was determined by random selection within each replication and the starvation times were recorded. Although the aphids were removed after the specified time periods, the plants were fumigated as a precautionary measure.

Results presented in table 1 indicate that as the test feeding time is increased, the number of infections increase quite rapidly from 5 to 20 seconds and then more slowly until a leveling off is indicated at 30 seconds. The results reveal that the inoculation threshold period lies somewhere between the 5- and 10-second feeding. No statistical analysis was done because of the definite trend of the data.

An analysis of variance was calculated to determine if randomness of starvation times equalized the distribution of this variable in the test. The F-value ($F = 0.47$, $F_{.01} = 3.51$) indicated that within this experiment, the starvation times were distributed equally.

When the results of the acquisition and inoculation threshold periods are considered, a point of interest arises that is noteworthy. Comparison of previously reported results of acquisition and inoculation threshold periods of other nonpersistent viruses with those of the severe isolate of the feathery mottle virus shows one overall difference. Previous workers have reported that it takes either a shorter or an equal time for an aphid to inoculate the virus than to acquire it. Reference to data presented in table 3 shows that the severe isolate of the feathery mottle virus is the reverse, for it takes an aphid less time to acquire the virus than it takes to inoculate it. At this time, these results may or may not be of fundamental significance and reasons for this reversal are not known.

Table 3.—Results of reports on acquisition and inoculation threshold periods of seven nonpersistent aphid-borne viruses as compared with the severe isolate of the feathery mottle virus.

VIRUS	AUTHORITY	ATP[a] (SECONDS)	ITP[a] (SECONDS)
Beet mosaic	Sylvester 1949	10	10
Brassica nigra	Sylvester 1950	10	5
Alfalfa mosaic (Strain of)	Swenson 1952	15–45	10–20
Henbane mosaic (Strain of)	Bradley 1952	5–10	5
Potato A	MacLachlan *et al.* 1953	15	10
Cabbage black ringspot	Hamlyn 1953	10	5
Lettuce mosaic	Sylvester 1955	10–15	10
Feathery mottle (Severe isolate of)	Author 1958	5	10

[a] Acquisition and inoculation threshold periods, respectively.

Table 4.—The effects of preacquisition and postacquisition starvation on the transmission efficiency of the severe isolate of the feathery mottle virus.[a]

STARVATION TIME (MINUTES)	PREACQUISITION	POSTACQUISITION
0	6/100	62/100
5	42/100	—
10	40/100	—
15	40/100	46/100
30	37/100	30/100
60	41/100	15/100
120	53/100	6/100
240	41/100	3/100
Check	1/150[b]	0/150

[a] Based on single apterous green peach aphids with 10 test plants per starvation time and a total of 10 replications.
[b] Probable greenhouse contamination.

PREACQUISITION STARVATION.—The relationships concerned with preacquisition starvation have been reported for many vector-virus combinations, and it has been fairly well established that preacquisition starvation prior to a short acquisition feeding will increase transmission efficiency of the vector with typical nonpersistent viruses (Watson & Roberts 1939, Sylvester 1953).

The purpose of the following investigation was to ascertain the preacquisition starvation relationships of the severe isolate of the feathery mottle virus and to determine if differences existed between this virus and other nonpersistent viruses. The experiment was designed to use single apterous green peach aphids for each test feeding with a series of 10 test plants per time period with 10 replications. In addition, 15 check plants were also used per replication. The preliminary starvation times, in minutes, were: 0, 5, 10, 15, 30, 60, 120, and 240. In an attempt to obtain precision in starvation, 10 envelopes were chosen for each time period and these were so marked. A single aphid was removed with a camel's-hair brush from a colony and placed in a leaf cage. The time for aphid feeding was written on the envelope and the cage placed within. After the starvation time had elapsed, each aphid was removed from the cage, placed on the source plant, and allowed to feed for 15 seconds, then artificially interrupted and moved to the test plant and left for 1 hour. For the 0-minute starvation, aphids were removed from the colony and placed immediately on the source plant. The time interval between removal from the cage and actual penetration by the aphids was not recorded, although observations suggested that as starvation time was increased, the time to make the initial puncture decreased up to 120 minutes starvation. After 240 minutes of starvation, the time before the aphid penetrated the leaf became slightly longer.

The data in table 4 indicate that the maximum transmission efficiency of the severe isolate of the feathery mottle virus may be obtained after 5 minutes of preacquisition starvation. From 0 to 5 minutes starvation, a marked increase in the number of infections occurs. Because of the rise at 120 minutes, a Chi square analysis was done to attempt to determine if this rise was significant ($\chi^2 = 6.41$, $df = 6$, $p = \approx 0.40$). From the results of the

analysis, support is given to the conclusion that the rise was perhaps a chance variation. This fact is further supported by the observation that in only 2 of the 10 replications was the 120-minute interval superior to the others, equal to in 3 additional and inferior to at least 1 of the other intervals in 5 out of 10 replications.

POSTACQUISITION STARVATION.—The effects of postacquisition starvation on transmission efficiency are not so well known as those related to preacquisition starvation. Some data have been published, however (Watson 1938, Sylvester 1954) which indicate that the efficiency of the vector for transmission decreases as postacquisition starvation increases.

Experiments were conducted using single apterous green peach aphids for each test period with a series of 10 test plants per time period with 10 replications. Fifteen check plants were used for each replication tested. The postacquisition starvation times, in minutes tested, were: 0, 15, 30, 60, 120, and 240. Aphids were removed from the colony, one at a time, at intervals of 5 minutes, each placed in leaf cages, and allowed to starve for 1 hour. Removal of single aphids at intervals made it possible to achieve relative uniformity in the preliminary 1-hour starvation. Each aphid was allowed to feed on the source plant for 15 seconds, artificially interrupted, and placed in a leaf cage for the designated starvation time. Each aphid was removed and then placed on the test plant to feed for 15 seconds to maintain uniformity of feeding, again artificially interrupted, and removed. The test plants were fumigated as a precautionary measure. No record was maintained as to the interval of time between removal of aphids after the preliminary 1-hour starvation and initial leaf penetration, nor after starvation for the tested times and initial penetration of the test plant.

The results presented in table 4 show that the postacquisition starvation relationships of this virus are similar to data presented by other workers on this phenomenon (Watson 1938, Sylvester 1954), in that, as the length of the postacquisition starvation period increases, the efficiency for transmission decreases. The data presented indicate that the rate of the loss of inoculativity is proportional to the amount of the virus in the aphid, or stated more simply, the virus has a half-life of approximately 30 minutes.

SERIAL TRANSMISSION.—Watson (1938) reported successful serial transmission of *Hyoscyamus* virus with aphids when she used short feeding periods on the test plants. Since this report, many other workers have been successful with serial transmission of nonpersistent aphid-borne viruses and there appears to be no common dispersal pattern (Bradley 1952, Sylvester 1954).

The following investigations were undertaken to determine if the severe isolate of the feathery mottle virus acted in a similar manner as that reported for other nonpersistent aphid-borne viruses. The experiment was designed to feed single aphids on a series of 10 plants per replication with a total of 50 replicated series. Five check plants were also used per series. To maintain equal preliminary starvation time for each series, single aphids were collected from a colony 30 minutes apart and placed in leaf cages to starve for 1 hour. In no case did the time to complete a series exceed 30 minutes. Single apterous green peach aphids were initially allowed to feed for 15 seconds on the source plant and then were artificially interrupted. The serial transfer was done in the same manner, that is, the aphid fed for 15 seconds on each test plant, was artificially interrupted, and then transferred to the next plant, and so on until completion of the series.

The results (table 5) show that 38 out of 50 insects (76%) were infective and these insects infected 115 out of 500 plants (23%). Each aphid fed on 10 plants, and therefore, if the assumption is made that the infective aphids were approximately equal in virus charge, the number of insects inoculating 0, 1, 2, . . . , 10 plants should follow a binomial distribution $(p+q)^{10}$ ($p=0.30$) assuming each infection as an independent and separate event. In addition, the assumption is made that the 12 aphids that did not transmit did not acquire the virus. Table 6 shows the results of calculations of the binomial. The calculated frequencies did not differ significantly from the observed ($\chi^2=4.07$, $df=4$, $p=\approx 0.30$), indicating the uniformity of virus charge for each individual.

As each series progressed, the number of infections declined and three aphids remained infective to the tenth plant fed upon.

Table 5.—Results of serial transmissions to show the distribution of infections with the severe isolate of the feathery mottle virus by 50 single apterous green peach aphids each fed on a series of 10 plants.

Successive Plants in the Test Feeding Series[a]										No. Infected	Pattern Frequency[b]
1	2	3	4	5	6	7	8	9	10		
+	+	−	+	−	+	+	−	−	−	5	1
+	+	−	+	−	+	−	−	−	−	4	1
+	+	−	+	−	−	−	+	−	−	4	2
+	+	−	−	+	−	+	−	+	−	5	1
+	+	−	−	−	−	+	−	−	−	3	1
+	+	−	−	−	−	−	+	−	−	3	1
+	+	−	0	−	−	−	−	−	+	3	1
+	−	+	+	−	−	−	+	−	−	4	2
+	−	+	−	+	+	−	−	−	−	4	1
+	−	+	−	+	−	+	+	−	−	5	1
+	−	+	−	+	−	−	−	−	−	3	1
+	−	+	−	−	+	−	−	+	+	5	1
+	−	+	−	−	+	−	−	+	−	4	1
+	−	−	+	−	−	−	+	−	−	3	1
+	−	−	+	−	−	−	−	−	−	2	3
+	−	−	−	+	−	+	−	−	−	3	1
+	−	−	−	−	+	−	−	−	−	2	1
+	−	−	−	−	−	−	+	−	−	2	1
+	−	−	−	−	−	−	−	−	−	1	3
−	+	+	−	+	−	+	−	−	−	4	1
−	+	+	−	+	−	−	−	−	−	3	4
−	+	+	−	−	−	+	−	−	−	3	1
−	+	+	−	−	−	−	−	−	+	3	1
−	+	−	+	+	+	−	−	−	−	4	1
−	+	−	+	−	−	+	−	−	−	3	1
−	+	−	−	+	−	−	−	+	−	3	1
−	+	−	+	−	−	−	−	−	−	2	1
−	+	−	−	−	−	−	−	−	−	1	2
											38

[a] (+) indicates infection; (−) no infections; (0) plant died. No infection resulted in 250 control plants.

[b] "Pattern Frequency" refers to the number of times that a transmission pattern occurred.

Table 6.—Tabulation of data on serial transmission of the severe isolate of the feathery mottle virus showing the distribution of infections in a series of 10 plants for each replication and 50 replications.

	Distribution of Infections	
	Expected	
Infections per Aphid	Binomial	Actual
0	1.1	0.0
1	4.6	5.0
2	8.7	6.0
3	10.3	14.0
4	7.6	9.0
5 or more	5.7	4.0

Calculations of the binomial presented by Bradley (1952), Sylvester (1955), and from those presented in this writing, indicate that each individual infective aphid acquires a similar charge of virus.

COMPARISON OF ARTIFICIALLY AND NATURALLY INTERRUPTED ACQUISITION FEEDINGS ON TRANSMISSION EFFICIENCY.—Since aphids were artificially interrupted in most experiments, it was thought desirable to determine if differences might exist in transmission efficiency between aphids which were artificially interrupted and those which were allowed to interrupt their feeding naturally. The effects of artificial interruption on transmission efficiency were first investigated by Bradley (1952). He showed that artificially interrupting the acquisition feeding of the aphid reduced the chance of obtaining an infection over that of natural interruption. He also investigated the effects of artificial interruption on test feedings and concluded that this did not seem to affect transmission efficiency. Sylvester (1954) supported the results reported by Bradley.

An experiment was designed to test if any effects on transmission efficiency could be related to artificial interruption of the acquisition feeding. Single apterous green peach aphids were aspirated from a colony at 5-minute intervals and each placed in leaf cages to starve for 1 hour. Following starvation, single aphids were allowed to feed on the virus source plant. The test was divided into two phases, one where the aphids were allowed natural interruption on the source, and the other, where the aphids were interrupted from their feeding artificially. Since, in previous experiments, the aphids were interrupted from their feeding at 15 seconds, this time was used for testing both phases. With artificially interrupted feedings, the 15-second point could easily be obtained. However, with natural interruption, it was somewhat more difficult. All aphids which naturally interrupted their feedings at 14 or 15 seconds were used, and all others discarded. After feeding, the aphids were placed on the test plants for 1 hour. The two phases were tested alternately, that is, one aphid was allowed natural interruption, the next, artificial interruption, and so on until one replication of 10 plants was completed for both phases. The test was continued until five replications were executed for each phase. For each replication, five check plants were also used.

Results of these tests indicate that little difference in transmission efficiency existed when aphids were either naturally or artificially interrupted (27/50 and 23/50) ((X^2) Chi = 0.64, df = 1, p = ≈0.40). The results appear to differ from those presented by Bradley (1952) and Sylvester (1954). Bradley, however, did not use the 15-second feeding time exclusively as was done here and thus the difference in results may be attributed to the feeding time variation. Sylvester (1954), working with the *Brassica nigra* virus, did use the 15-second feeding time for comparison of natural and artificial interruption and his data showed that transmission efficiency was reduced when artificial interruption was used (17/45 and 9/45). The variation of results may be attributed to a difference in the vector-virus relationships between the two viruses.

VECTOR RANGE.—The ability of many aphid species to act as vectors of nonpersistent viruses has been reported by several workers and examples are: western celery mosaic (Severin & Freitag 1938), onion yellow dwarf (Tate 1940), and bean yellow mosaic (Swenson 1957.) Probably the most striking example of this ability with nonpersistent viruses is that of the onion yellow dwarf virus where 48 of 51 aphid species tested were reported to be vectors (Tate 1940).

The following experiments were undertaken to test several aphid species for their ability to transmit the severe isolate of the feathery mottle virus. Nine species of aphids, including the green peach aphid, were chosen from available greenhouse colonies at Berkeley. The aphid species tested and the plants on which they were reared are as follows: green peach aphid on mustard; celery aphid, *Aphis apii* Theob., on celery; cabbage aphid, *Brevicoryne brassicae* (Linn.), on mustard; spotted alfalfa aphid, *Therioaphis maculata* (Buckton), on alfalfa; pea aphid, *Macrosiphum pisi* (Harr.), on broad bean; potato aphid, *Macrosiphum solanifolii* (Ashm.), on potato; melon aphid, *Aphis gossypii* Glover, on cucumber; yellow willow aphid, *Cavariella aegopodii* (Scopoli), on celery; and bean aphid, *Aphis fabae* Scopoli, on sugar beet.

The experiment was designed to use 10 plants per replication for each species tested with 10 replications and 15 check plants per replication. All aphid species were tested at the same time per replication to maintain uniformity for comparison. Single aphids of each species were collected at 5-minute intervals in separate leaf cages and each starved for 1 hour. Aphid species were collected at random and consequently fed at random in order to provide for more uniformity. Following the preacquisition starvation, single aphids were placed on the source plant and allowed to feed for 15 seconds and then were artificially interrupted. When each series had been fed, the collection was repeated until 10 aphids were fed for each aphid species tested and this made up one replication. The aphids were placed on the test plant for 1 hour, after which all plants were fumigated.

The results in table 7 show rather conclusively that, in addition to *Myzus persicae*, *Aphis apii* and *Aphis gossypii* are also vectors of the severe isolate of the feathery mottle virus, although somewhat less efficient. The results of these trials also indicate that the severe isolate of the feathery mottle virus appears to have a restricted vector range since six aphid species of nine tested failed to transmit the virus. These results appear to differ with most of those previously reported by other workers on

Table 7.—Results of trials conducted to ascertain the ability of some aphid species to transmit the severe isolate of the feathery mottle virus.

Species Tested	No. Infected / No. Tested[a]
Myzus persicae	67/100
Aphis gossypii	51/100
Aphis apii	38/100
Brevicoryne brassicae	0/100
Therioaphis maculata	0/100
Macrosiphum pisi	0/100
Macrosiphum solanifolii	0/100
Cavariella aegopodii	0/100
Aphis fabae	0/100
Check	0/150

[a] Based on 10 test plants for each species tested with 10 replications.

aphid-borne nonpersistent viruses. It would appear, therefore, that the vector range of the severe isolate of the feathery mottle virus is much more restricted than that reported for such viruses as onion yellow dwarf, western celery mosaic, bean yellow mosaic, and several others. If the number of tests were increased, or if other aphid species were tested, others might be found that could transmit the virus.

It is also of interest to note from the results in table 7 that of the three species of aphids that did act as vectors of the severe isolate of the feathery mottle virus, all did so with reasonably high efficiencies (67%, 51%, 38%). This is another point of difference concerned with vectors of nonpersistent viruses, since previous reports have indicated that efficiencies can vary from low to high with a given virus and several aphid species (Sylvester & Simons 1951, Swenson 1957).

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Relationship between Metabolism and Differential Toxicity of Malathion in Insects and Mice[1]

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ABSTRACT

An attempt has been made to account for the selective toxicity of malathion on the basis of differences in its metabolism by various species. Eleven metabolites were found in the German cockroach (*Blattella germanica* (L.)), and American cockroach (*Periplaneta americana* (L.), and house fly (*Musca domestica* L.), seven in the mouse. Most metabolites were identified. Degradation of malathion is much more extensive in the mouse than in the insect and malaoxon production is correspondingly lower; these effects account satisfactorily for the low toxicity of malathion to the mouse. The low toxicity of topically applied malathion to the German cockroach is attributable to poor penetration through the integument.

Malathion (*O,O*-dimethyl *S*-(1,2-bis-carboethoxy) ethyl phosphorodithioate) has attracted considerable interest in recent years because it is highly toxic to insects and relatively nontoxic to mammals (table 1). March *et al.* (1956) suggested that the metabolism of malathion in the American cockroach, *Periplaneta americana* (L.), is apparently less extensive than in the mouse or chicken since only two metabolites were found in the cockroach gut, whereas seven were found in the excreta of mice and chickens. The paper chromatographic separation of the malathion metabolites as used by March *et al.* did not give quantitative data, nor were the metabolites identified.

O'Brien (1957) studied the *in vitro* oxidative (activating) and degradative (hydrolytic) activities of various tissues of the mouse and American cockroach, and found the balance of the two metabolic mechanisms to correlate with the toxicity data. Recent advances in the synthesis of radioactive organophosphates of high specific activity (Casida 1958) coupled with improved methods for the chromatographic separation of the metabolites (Plapp & Casida 1958) has facilitated insect metabolism studies at dosage levels corresponding to the LD_{50} or less.

The present study was initiated to establish on a quantitative and qualitative basis the metabolism of malathion in the American cockroach, German cockroach (*Blattella germanica* (L.)), house fly (*Musca domestica* L.) and mouse, and to determine the extent to which metabolic differences could account for the selective toxicity. The selection of the two species of cockroach was based on their rather unusual difference in topical LD_{50} values as shown in table 1 (Krueger & Casida 1957).

Table 1.—Toxicity of malathion and malaoxon to several insects and mice.[a]

SPECIES	MALATHION LD_{50}(γ/GM.)	MALAOXON LD_{50}(γ/GM.)
American cockroach	16	1.5
German cockroach	120	15
House fly	30	15
White mouse	1,590	75

[a] Insects treated topically, mice injected intraperitoneally.

The toxicity of malathion is probably due to the production in the body of malaoxon, *O,O*-dimethyl *S*-(1,2-bis-carboethoxy) ethyl phosphorothiolate, which is more potent as an anticholinesterase by a factor of between 700 and 2,000, depending upon cholinesterase source (March *et al.* 1956, O'Brien 1957). However, malaoxon production *in vivo*, although inferred, has never been unambiguously demonstrated. If the selective toxicity of malathion is due to differences in metabolism of the organophosphate by the various species, higher levels of malaoxon in susceptible species, after poisoning, should be found.

METHODS: *Synthesis of P^{32} Malathion.*—One hundred mc. of $H^3PO_4^{32}$ (which was obtained in HCl solution) were evaporated with dry air to dryness in a round-bottom flask. Then 0.25 gram of P_2S_5 was added, a condenser attached and the flask gassed with dry CO_2. The contents were heated gently in the beginning and then vigorously for 10 additional minutes. On cooling, yellow crystals form which are $P_2{}^{32}S_5$ (Casida 1958). Five ml. of toluene and a stirring bar were then added to the reaction flask, the condenser replaced, and 0.2 ml. of anhydrous methanol dissolved in 2 ml. toluene added dropwise. The contents were refluxed between 80 to 90° C. until all the $P_2{}^{32}S_5$ had reacted. The temperature was dropped to 50 to 60° C. and 0.425 ml. diethyl maleate was added with 3 ml. toluene. The mixture was then refluxed at 85° C. with stirring for 4 hours. The residue was concentrated by evaporation and then dissolved in $CHCl_3$ and washed with an equal amount of 10% Na_2CO_3. The compound was then purified by partition chromatography on a Celite column with *iso*octane-methanol as solvents (Bowman & Casida 1957). The product formed in 70% yield with an activity of 15,000 cpm/gamma. The radioactive material was identical in infrared spectrum to known malathion.

The oxygen analog of malathion (malaoxon) (n_D^{25} 1.4655, B.P. 138–140° C. at 0.001 mm.) was kindly supplied by American Cyanamid Corporation.

Treatments.—The insect strains, rearing procedures, and topical application techniques were identical to those described in a previous paper (Krueger & Casida 1957). Injections were made with a micrometer-driven syringe fitted with a No. 27 hypodermic needle. Propylene glycol was used as the carrier solvent. The American cockroach was injected with 10 μl. at the femoro-tibial joint. For the German cockroach 1.3 μl. were injected into the intersegmental membrane separating the fourth and fifth

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abdominal sternites on the right side. After the dose was delivered, the insect was left on the needle for about 10 seconds to relieve back pressure and consequent loss of injected dose by bleeding. Mice (12-week old females from Taconic Farms, Germantown, New York) were injected intraperitoneally with 0.2 ml.

Extraction Procedures.—The topically treated insects were rinsed with 50 ml. of acetone to remove unabsorbed malathion remaining on the surface of the insect. Four grams of insects were homogenized in a Lourde's Multimixer with 80 ml. of chloroform and 80 ml. of water containing a sufficient quantity of trichloroacetic acid to lower the pH of the homogenate to pH 2.5. The homogenate was then filtered through celite in a Buchner funnel, and the precipitate was washed on another filtering flask with 40 ml. each of methanol and acetone, the filtrate evaporated to dryness and taken up in the chloroform-water filtrate, which was centrifuged and the water layer removed. The chloroform was repartitioned against water containing enough NaOH to give a pH after partitioning of 7 to 7.5 and the aqueous fractions were combined. Individual 40-gm. mice were homogenized as above and extracted, using identical materials as those used for the insect studies, except that twice the amount of solvent was used throughout the analysis. With the above extraction procedure 90 to 95% of known amounts of added malathion could be recovered in the chloroform from the various preparations analyzed.

Column Chromatography.—Adsorption chromatography was used in the identification of the chloroform-soluble compounds malathion and malaoxon. To aluminum oxide (Woelm, acid activity grade 1 for chromatographic analysis) was added 2% by weight of water, and the hydrated aluminum oxide was immediately suspended in Skellysolve B and packed into a 10 mm.×170 mm. column. The chloroform soluble sample was evaporated to dryness, taken up in 16 ml. of Skellysolve B and poured onto the column. Malathion and malaoxon were not eluted from this column with Skellysolve B. Malathion was eluted with 200 ml. of benzene, and malaoxon with 150 ml. of chloroform.

Total recovery was not realized using this procedure. However, experiments showed that proportion of malathion and malaoxon which could not be eluted from the aluminum oxide (approx. 25%) was in the same ratio (P=S/P=O) as that in the original sample. Corrections were made for the loss in calculating the amounts of metabolites recovered throughout this investigation.

For the water-soluble metabolites an ion exchange chromatography system, as described by Plapp & Casida (1958), was used with slight modifications. The elution gradients were modified to improve separation of the water-soluble metabolites unique to malathion metabolism (fig. 1). Known water-soluble metabolites, kindly supplied by American Cyanamid Corporation, were cochromatographed and located by phosphorus analysis (Casida *et al.* 1952). Radioactive measurements were made by previously described procedures (Casida *et al.* 1956).

RESULTS.—*Topical Treatments.*—Experiments were only carried out at doses and times for which all animals survived. For each insect two doses of P^{32}-malathion were used, of which the higher was 10 times the lower, and the lower was well below the LD_{50}. House flies topically treated with 150 γ/g. ($5\times LD_{50}$) survived only 4 hours after treatment. At the higher dosage level all the insects studied exhibited typical organophosphate symptomatology 0.5 to 1 hour after treatment. The astonishingly long time to death which had previously been observed for the American cockroach (O'Brien 1956) was confirmed. Data for the treatment of mice at 30 γ/gm. are also included; however, particular note should be made of the fact that the mice were treated by injection.

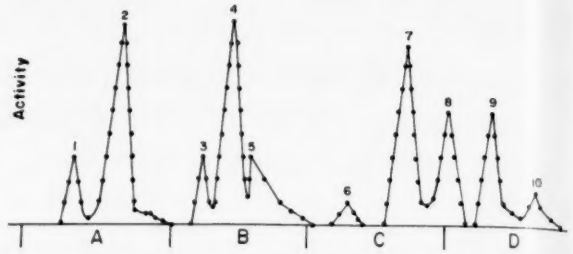

FIG. 1.—Ion exchange separation on Dowex 1 of water-soluble metabolites of malathion. A series of gradient elutions were used, in each case 500 ml. each of the following pairs A: HCl pH 1.5/HCl pHl. B. HCl pH2+methanol (1+4)/HCl pHl+methanol (1+4). C: HCl pHl+methanol (1+3)/HCl 1N+methanol (1+3). D: HCl 1N+acetone (1+3)/HCl conc.+H_2O+acetone (1+1+6).

Table 2 shows that the rates of disappearance of malathion from the surface of the insect were dependent on the quantity of malathion applied and on the time after treatment, with little apparent differences appearing between the insects studied. Generally, the disappearance rates were very rapid, with 70 to 94% of the applied dose disappearing within 4 hours after a 0.5 LD_{50} dose. At 0.5 hour a 10-fold increase in dose level resulted in a 3- to 4-times reduction in the per cent disappearance. This phenomenon is probably caused, in part, by the decreased solvent effect as the amount of malathion is increased for

Table 2.—The absorption, degradation and activation of P^{32}-malathion in insects and mice.[a]

Hours After Treatment	Dose γ/Gm.	Per Cent of Applied Dose Absorbed	Per Cent of Absorbed as: H_2O Solubles	$CHCl_3$ Solubles	Unextractable	γ/Gm. Malaoxon
American cockroach						
0.5	4	54	39	56	5	0.036
4	4	94	43	42	15	.079
24	4	97	56	26	18	.040
0.5	40	22	6	91	4	.28
4	40	70	41	49	10	.22
24	40	93	75	14	11	.19
German cockroach						
0.5	60	34	11	84	5	0.79
4	60	83	38	53	9	.58
24	60	88	72	18	10	.47
0.5	600	14	6	91	3	3.0
4	600	44	38	50	12	2.6
24	600	80	54	39	7	1.9
House fly						
0.5	15	47	23	68	9	0.17
4	15	70	56	30	14	.22
24	15	86	66	10	24	.039
0.5	150	13	19	70	11	.19
4	150	40	51	39	10	.14
Mouse						
0.5	30	—	70	20	10	.13

[a] Topical application to insects, intraperitoneal injection into mice.

a given volume of acetone. By 24 hours disappearance was almost complete for both doses.

No attempt was made to determine the distribution of malathion or its metabolites in the tissues of insects or mice. It is reasonable to assume that an analysis of the whole animal would give a total quantitative picture of the metabolic processes involved in the various species.

Preliminary experiments, in which labeled malathion was added to homogenates, showed that malathion could not be extracted by chloroform from neutral or HCl-treated tissue homogenates, but could be almost completely extracted if the homogenate was treated with trichloroacetic acid. The failure to extract malathion from nonprecipitated tissue homogenates is probably attributable to protein binding and the possible emulsion characteristic associated with water-soluble protein (Krueger *et al.* 1959). The protein residue with the standard (trichloroacetic precipitation) procedure contained varying amounts of unextractable malathion or its metabolites, to an extent increasing with longer periods after treatment (table 2).

Table 2 shows that in general the per cent of the absorbed dose hydrolyzed at any time was the same at both dosage levels. This result means (a) that the hydrolysis rate was not directly proportional to internal malathion level, since the internal malathion level is given by dose applied $\times$ fraction absorbed, and the latter varied; (b) that the hydrolyzing enzymes were probably not saturated at the highest dosages used. At either 0.5 or 4 hours, about a 7-fold difference in the absolute level of chloroform solubles was associated with the 10-fold difference in applied dose.

The levels of malaoxon do not appear to be directly proportional to the dosages or to the total chloroform solubles present at the time of analysis. The poor correlation of chloroform solubles with the levels of malaoxon implies that at any given dose, the rates of formation and degradation of malaoxon are about the same. In the case of the house fly only, the malaoxon level is similar at both doses, suggesting that the oxidizing system is saturated even at the low dose.

Clearly malathion degradation in the mouse is much more rapid than in the insect: 70 to 80% is degraded in 0.5 hour. This factor will be discussed in detail below.

An example of the separation of water-soluble metabolites is given in figure 1 for the house fly 4 hours after 150 γ/gm. topical dose of P^{32}-malathion. Table 3 shows the levels and partial identification of the 10 water-soluble metabolites that appeared for the various treatments. It is apparent from these results that the metabolism of malathion in insects involves at least two major degradative pathways: that owing to phosphatases attacking P-S-C ('P' in fig. 2) and that owing to carboxy esterase(s) attacking $COOC_2H_5$ ('C' in fig. 2) both of which systems contribute substantially to the initial hydrolysis of the molecule. Carboxyesterase action predominates in the mouse, phosphatase in the house fly; in the cockroach both are about equal. The hydrolysis products which appear 4 hours and 24 hours after treatment may represent secondary cleavage of one or more of the original hydrolytic metabolites; this fact is illustrated by the increase of phosphoric acid as a function of time after treatment. The levels of phosphatase products

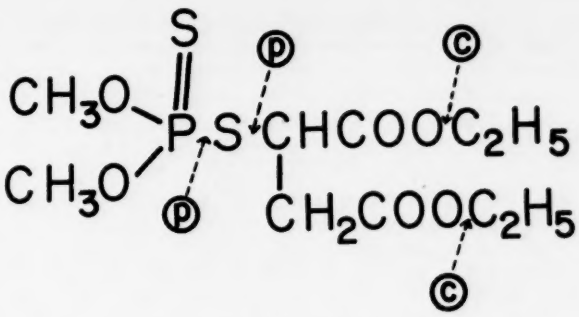

FIG. 2.—Arrows indicate hydrolysis by phosphatases (p) or carboxyesterases (c).

generally increase as the length of time after treatment increases. The phenomenon is probably due to secondary cleavage of either the monoesters or diacid of malathion (formula in table 3). The carboxyesterase degradation in all the animals studied resulted predominantly in the monoester of malathion.

In any one species the pattern of water-soluble metabolites does not vary much between the two dose levels, suggesting that no saturation of any one system is achieved.

The data presented so far threw no light on the intriguing difference between LD_{50}'s of the two cockroach species. It was felt that comparison at a single dose level might give more directly comparable data.

Treatments by Injection.—In the subsequent studies, two comparisons were made: of the German versus the American rockroach, and of the mouse versus the American cockroach. In both cases, 30 γ/gm. of malathion were injected (in order to eliminate variations in penetration), and the levels of water-solubles, total chloroform-solubles and malaoxon were studied as a function of time.

Figures 3 and 4 show data for the two cockroaches. In the American cockroach a small but consistently higher level of chloroform-soluble products existed, with a corresponding lower level of water-solubles. The malaoxon levels were very similar, with a somewhat higher

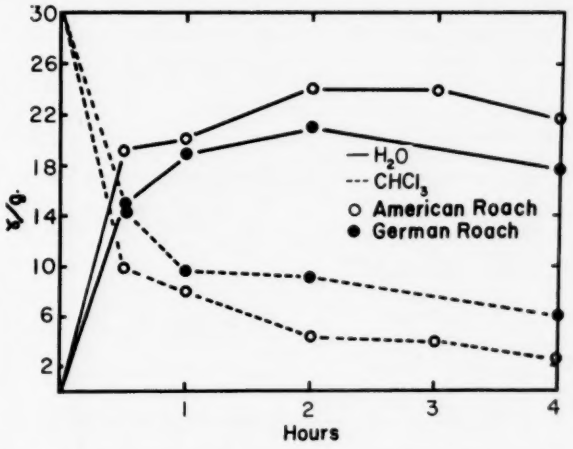

FIG. 3.—Chloroform and water-soluble products in the American cockroach and German cockroach after injection of 30γ/gm. of P^{32}-malathion.

Table 3.—Nature of water-soluble metabolites of P^{32}-malathion based on ion exchange chromatography.[a]

Hours After Treatment	Dose γ/Gm.	Per Cent as Various Hydrolysis Products: $(OH)_2P(O)OH$ Peak 1	$(MeO)_2P(O)OH$ Peak 2	$(MeO)_2P(S)OH$ Peak 7	$(MeO)_2P(S)SH$ Peak 10	Total Phosphatase Products	Mono Ester[b] Peak 4	Diacid[c] Peak 5	Total Carboxyesterase Products	Peak 3[d]	Peak 6	Peak 8	Peak 9	Total Unknowns
					American cockroach									
0.5	4	4.6	6.4	12	5.6	29	42	8.2	50.2	3.8	4.0	7.7	5.7	21.2
4	4	1.0	5.8	22	1.8	31	48	0	48	0.3	0.3	14	6.4	21
24	4	4.2	19	27	0	50	44	0	44	0	0	0	6.1	6.1
0.5	40	0	2.0	27	3.0	32	43	0	43	13	0	5	7.0	25
4	40	1.7	12	26	3.4	43	16	8.4	24.4	1.7	1.8	15	14	32.5
24	40	4.7	18	19	0	42	17	11	28	0.6	0	9	21	30.6
					German cockroach									
0.5	60	0	8.0	22	14	44	43	0	43	3.0	2	5	3.0	13
4	60	1.0	14	25	4.7	45	21	14	35	0	0	10	10	20
24	60	3.9	18	25	0	47	17	19	36	0	0	0	8.0	17
0.5	600	0	16	25	0	41	28	6	34	9.0	0.4	11	5	25.4
4	600	1	17	21	2	41	27	9	36	2.0	2.0	11	8	23
24	600	6.6	9.4	26	0	42	16	7.3	23.3	0.5	1.2	11	22	34.7
					House fly									
0.5	15	0	7.0	36	6	49	27	0	27	4.8	7.4	5.0	6.8	24
4	15	2.0	31	35	0	68	10	0	10	2.0	12	4.7	2.8	21.5
24	15	5.0	20	42	0	67	10	2	12	2.0	9	0	10	21
0.5	150	3.0	20	37	0	60	20	4.2	24.2	3.4	10	0	2.4	15.8
4	150	4.0	22	33	0	59	3.1	2.3	5.4	1.2	14	12	8.4	35.6
					Mouse									
0.5	30	0	2.5	13	5	20.5	68	0	68	1.5	0	0	10	11.5

[a] Topical application to insects, intraperitoneal injection into mice.

[b] $(CH_3O)_2PSCHCOOC_2H_5$ (CH$_2$COOH)

[c] $(CH_3O)_2PSCHCOOH$ (CH$_2$COOH)

[d] Peaks 3, 6, 8 and 9 were not identified

level appearing at 1 hour in the German cockroach. These small differences hardly appear adequate to account for the 15-fold difference in LD_{50}.

Although the data upon surface disappearance were not different for the German and American cockroaches, there was a possibility that disappearance from the surface could not be equated with penetration into the body. The LD_{50} of malathion was therefore determined by injection directly into the body. The resulting LD_{50}'s were 7.2 γ/gm. for the American, 8.0 for the German cockroach. The selectivity shown with topical application can, therefore, be due only to penetrative differences. It also follows that the surface disappearance data gives no evidence as to penetration into the body, and may measure only penetration into the integument.

Figures 5 and 6 show data for the mouse and American cockroach. The chloroform-soluble levels fell more rapidly in the mouse. The water-solubles were at first very similar in level, but at 1 hour they declined precipitously in the mouse, presumably owing to urinary excretion. In the cockroach little excretion occurs; this may be due, in

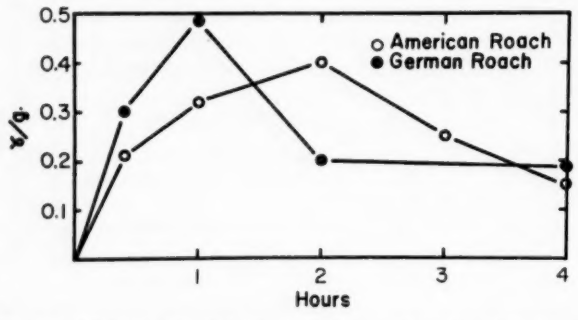

FIG. 4.—Malaoxon levels in the American cockroach and German cockroach after injection of 30γ/gm. of P^{32}-malathion.

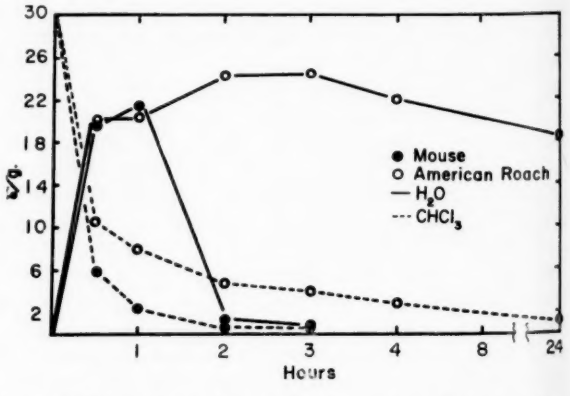

FIG. 5.—Chloroform and water-soluble products in the American cockroach and mouse after injection of 30γ/gm. of P^{32}-malathion.

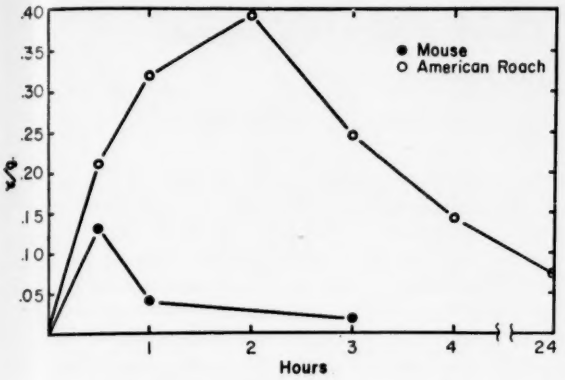

FIG. 6.—Malaoxon levels in the American cockroach and mouse after injection of 30γ/gm. of P^{32}-malathion.

part, to the lesser efficiency of insect excretion, but is probably largely caused by the paralysis of the cockroach.

A very striking difference was seen (fig. 6) in the malaoxon levels for the two animals: in the cockroach the level was far higher and maintained far longer than in the mouse. There is approximately a 10-fold difference in the area under the two curves of figure 6. This striking difference is probably quite sufficient to account for the difference in LD_{50}'s. Such a difference could be caused by metabolic or excretory effects. Since unhydrolyzed organophosphates are excreted in urine to a negligible extent (Dauterman *et al.* 1959, Casida *et al.* 1958), the difference must be due to a metabolic effect.

DISCUSSION.—When an animal is poisoned, the following processes may occur: penetration, metabolism, excretion, storage, approach to and attack on target, and physiological consequences of attacked target. If a poison selects between two species, there must be a difference in one or more of these steps. For malathion two totally different types of selectivity have been shown: penetrative, with respect to the two cockroaches; and metabolic, with respect to the American cockroach and house fly as compared with the mouse. This metabolic effect could take the form of particularly low oxidation of malathion to malaoxon in the mouse, or of particularly extensive degradation of malathion or malaoxon in the mouse. In view of the high carboxyesterase activity of the mouse, and since mouse tissues degrade malaoxon much more rapidly than insect tissues (O'Brien 1957), the metabolic effect is probably caused by extensive degradation.

A possible physiological consequence of the paralysis resulting from organophosphate poisoning might be dehydration. However, brief experiments have indicated that the LD_{50} of malathion for the American cockroach is not significantly altered by exposure either to air dried over calcium chloride or to water-saturated air.

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Different Concentrations of Thiodan for the Control of Cotton Insects[1]

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ABSTRACT

Three small-plot experiments were conducted to evaluate the effectiveness of Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) in the control of cotton fleahopper (*Psallus seriatus* (Reut.)), bollworm (*Heliothis zea* (Boddie)), cabbage looper (*Trichoplusia ni* (Hbn.)) and boll weevil (*Anthonomus grandis* Boh.) Dust formulations were used for the first and third experiments; sprays for the second. Thiodan 3% and 4% dust as well as sprays containing 0.52 and 0.32 kilograms of technical Thiodan per hectare gave good results compared with other commonly used insecticides.

Three small-plot experiments were conducted during the summer of 1958 to evaluate the effectiveness of Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) in the control of different cotton insects. Two of these experiments were located at the El Alto farm, Matamoros, Tamps.[2] and one at the Agricultural Experiment Station, Apodaca, Neuvo Leon.

The experimental plots at the El Alto farm presented rather light infestations of cotton fleahopper (*Psallus seriatus* Reut.), bollworm (*Heliothis zea* (Boddie)) and cabbage looper (*Trichoplusia ni* (Hbn.)). The experiment at Apodaca was conducted only against the boll weevil (*Anthonomus grandis* Boh.

Experiment 1.—The aim of this experiment was to compare three Thiodan dust formulations (2.5%, 3% and 4%, each containing 40% sulphur) with the commonly used insecticides and concentrations of that area. These insecticides were toxaphene 20%, Folidol 1.5% (methyl parathion), and dieldrin 2.5%, all in dust form.

A randomized plot arrangement with four replicates was used including four untreated plots for a total of 28 plots. Each plot was 10 rows wide and 30 meters long. The insecticides were applied by means of rotary hand dusters with a total of five applications, as follows: May 7 and 22, June 16 and 25, July 2. Applications were not made weekly because of adverse conditions, owing to heavy rainfall during the season. The aim was to try to apply 20 kgs. per hectare (approximately 20 lbs. per acre) but owing to the usually wet ground this amount was often exceeded. Infestation counts were made 3 days after each application. Counts of cotton fleahoppers, bollworm damaged squares, and bolls were made in the usual manner. For cabbage looper infestation the number of worms on plants per 15 mts. row were counted; yield data were taken from the whole plots. All data were analyzed and the significant difference (D) was calculated using Tukey's method (Snedecor 1957, p. 251).

In table 1 means of the data taken for the three cotton insects, yield of seed-cotton and amount of dust, as well as technical material, are presented. Results indicate that Thiodan 3% and 4% significantly reduced fleahopper population as compared with check plots, but there was no significant difference among untreated plots, and those treated with Thiodan 2.5% and the other three insecticides. All the insecticide treatments reduced significantly the per cent bollworm damaged squares but did not show significant difference among them. The overall per cent bollworm-infested bolls was considerably higher than the damaged squares and again all treated plots presented significantly less bollworm damage than the untreated plots. Damaged bolls were significantly lower in plots treated with Thiodan 4% than in plots treated with Thiodan 2.5% and 3% as well as with dieldrin. These last mentioned compounds gave results similar to treatment with toxaphene and Folidol. Significantly more cabbage loopers were counted on plants of untreated plots and again Thiodan 4% gave best control, significantly better than toxaphene, Thiodan 2.5%, or dieldrin.

No yield differences were observed in the statistical analysis.

Experiment 2.—This second experiment had the main purpose of comparing Thiodan spray at 0.52, 0.32 and 0.21 kgs. of technical material per hectare with the commonly used insecticides and concentrations used in the Matamoros area. The basic Thiodan material was a 17.5% emulsion. The other insecticides were toxaphene, Folidol, and endrin at 2.2, 0.72 and 0.33 kgs. of technical material per hectare, respectively.

The randomized plot arrangement included four untreated plots; each plot was 10 rows wide and 20 meters long. The insecticides were applied by means of pressure sprayers with a capacity of 16 liters. This amount of spray was enough to cover the four replicates during the first 2 applications, but 32 liters had to be used for the subsequent 3 applications, maintaining constant the technical material per hectare. Application dates were May 8 and 23, June 17 and 27, and July 2.

Table 2 shows the results obtained for the three cotton insects present and the yield of seed cotton per hectare. When comparing the means for cotton fleahopper control all insecticides, with the exception of endrin, were significantly efficient in controlling this pest. The highest concentration of Thiodan as well as Folidol and toxaphene were significantly better than endrin. No difference was evident between this latter compound and Thiodan 0.21 kgs. per hectare, or among the remaining treatments. Per cent bollworm damaged squares was significantly lower in treated plots with the exception of the lowest concentration of Thiodan; otherwise there was no difference in the effectiveness of the insecticides. The untreated plots showed significantly more damaged bolls and Thiodan 0.52 kgs. of technical material per hectare resulted in significantly better control than Thiodan 0.21, toxaphene, or endrin. Thiodan 0.32, Folidol, and endrin were superior to Thiodan 0.21 and there was no

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[2] In cooperation with Compañía Química Hoechst de México, S. A. The technical-assistance of Ing. J. L. Purata is gratefully acknowledged.

Table 1.—Means of data taken in cotton insect dust experiment, Matamoros, Tampaulipas.

Treatments[a]		No. of Flea-hoppers	Bollworm Damaged Squares (%)	Bollworm Damaged Bolls (%)	No. of Cabbage Loopers on 15 Meters	Seed Cotton per Hectare (Kg.)	Dust Applied per Hectare per Application (Kg.)
Thiodan	4	2	0.7	6.0	2.5	1,611	25.1
	3	3	1.5	9.5	4.8	1,282	27.1
	2.5	5.5	1.5	11.3	5.6	1,364	26.5
Folidol	1.5	5	2.7	8.3	4.5	1,659	23.1
Toxaphene	20	7.5	2.2	9.2	6.2	1,456	27.9
Dieldrin	2.5	8.5	1.7	9.5	5.2	1,258	24.6
Untreated		15	5.5	18.8	9.5	1,216	—
L.S.D. (Tukey's method)		11.4	2.2	3.4	2.4	ns	—

[a] Figures following the insecticide are per cent of technical material in finished dust.

Table 2.—Means of data taken in cotton insect spray experiment. Matamoros, Tampaulipas.

Treatments[a]		No. of Flea-hoppers	Bollworm Damaged Squares (%)	Bollworm Damaged Bolls (%)	No. of Cabbage Loopers per 15 Meters	Seed Cotton per Treatment (Kg.)	Seed Cotton per Hectare (Kg.)
Thiodan	0.52	0.8	1.5	6.0	3.0	42.2	2,110
	0.32	1.8	1.8	7.8	4.0	33.2	1,657
	0.21	2.3	2.1	12.2	5.1	27.8	1,391
Folidol	0.72	1.3	1.8	8.1	4.0	36.7	1,836
Toxaphene	2.20	1.5	1.3	10.3	5.6	27.1	1,352
Endrin	0.33	4.8	1.5	9.0	4.1	29.8	1,460
Untreated		7.0	5.0	20.2	9.3	23.5	1,277
L.S.D. (Tukey's method)		2.7	3.0	2.8	2.5	14.1	—

[a] Figures following the insecticides are average number of kilograms of technical material applied per hectare per application—5 applications were made.

Table 3.—Boll weevil punctured squares and average number of kilograms of dust applied per hectare. Apodaca, Nuevo Leon.

Treatments[a]	Mean Per Cent Punctured Squares (Angles)[b]	Kilograms of Dust/Applied per Hectare (Average)[c]
Thiodan 4%	36.7	25.8
3%	36.9	23.3
Dieldrin-DDT (2.5–10%)	40.0	20.0
Toxaphene 20%	41.8	23.0
BHC-DDT (3–5%)	44.7	25.5
Untreated	53.6	—
L.S.D. (Tukey's method)	6.7	—

[a] All treatments except dieldrin-DDT were formulated to contain 40% of sulfur.
[b] Average of 5 weekly counts.
[c] Average of 12 applications.

difference between the values obtained for Folidol and Thiodan at 0.33 and 0.52 kgs. of technical material per hectare, respectively. All insecticides significantly reduced looper infestation; the difference between Thiodan 0.52 and the other compounds was not great enough to be significant. Plots treated with Thiodan 0.52, 0.32, and Folidol each yielded significantly more seed cotton than untreated plots; there was no difference among insecticides.

Experiment 3.—This experiment was located at the Agricultural Experiment Station and was designed to compare Thiodan 3% and 4% dust plus 40% sulfur, toxaphene 20% plus 40% sulfur, BHC 3%-DDT 5%-sulfur 40% and a dieldrin-DDT mixture (2.5-10%). The randomized plot arrangement had five replicates and included five untreated plots. Each plot was eight rows wide and 20 meters long and the replicates were separated from each other by spaces three meters wide. Insecticides were applied by rotary hand dusters and a total of 12 applications were made at 4- and 5-day intervals from July 10 until August 29. Applications were started rather late, since weevil infestation did not appear until June, increasing very rapidly. Heavy rains during the latter part of June and July made applications impossible before the second week of July, when the infestation reached an average of 76% punctured squares. For the statistical analysis per cents were transformed into angles.

In table 3, per cent punctured squares in angles, as well as amount of dust applied (kgs. per hectare) are shown. Results indicate that all treated plots had significantly less puctured squares than untreated plots. Thiodan 3% and 4% were significantly more efficient than BHC-DDT-S, while toxaphene and dieldrin-DDT did not show significant differences with other insecticides used. Yield data were not taken, because of almost continuous rain during September.

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The Release of a Neuroactive Agent by the American Cockroach after Exposure to DDT or Electrical Stimulation[1]

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ABSTRACT

A neuroactive substance is released into the blood of American cockroaches (*Periplaneta americana* (L.)), during the course of DDT poisoning. The active principle is soluble in water, methanol, and ethanol, but insoluble in nonpolar solvents. Biological activity of the toxicant is destroyed by acetone and alkaline conditions. Although the chemical structure of the toxicant is unknown, it is chemically, chromatographically, and biologically distinct from acetylcholine, epinephrine, norepinephrine, histamine, γ-aminobutyric acid and 5-hydroxytryptamine. A substance with the same biological, chromatographic, and chemical properties as the DDT-induced agent can be produced in cockroaches by repeated mild electrical shocks. Electrical stimulation of isolated thoracic and abdominal nerve cords also results in the release of the substance into the perfusing saline, as does topical application of DDT to the intact cerci of an isolated central nerve cord. The source of the toxicant is apparently the central nervous system itself during periods of great nervous activity, whether initiated by electrical stimulation or by constant bombardment of sensory-central synapses due to excessive afferent impulses generated in the sensory nerves by direct action of DDT.

In 1946 Roeder & Weiant reported that the site of action of DDT was the sensory nervous system of the insect. Concentrations of DDT as low as 0.01 p.p.m. induced instability in afferent nerves typified by the appearance of ascending high frequency trains of impulses. They found that DDT had no significant direct action on either association neurons of the central nervous system or on motor neurons. Welsh & Gordon (1947), working with crayfish, confirmed this observation and stated that the primary action of DDT was to cause an increase of afferent discharges, leading to hyperactivity and eventual reflex incoordination.

In 1952 Sternburg and Kearns reported the presence of a toxic substance in the blood of American cockroaches (*Periplaneta americana* (L.)) poisoned by DDT. The direct action of the toxicant on the central nervous system of the cockroach, and its toxic action when injected into DDT-resistant house flies (*Musca domestica* L.) indicated that it was not DDT. They confirmed this by chemical analysis of toxic blood, which was found to contain only traces of DDT, in amounts far below that required to affect resistant house flies. Subsequent work on this problem is largely unpublished, except for a brief abstract of a paper presented to the American Physiological Society, April 18, 1957 (Sternburg *et al.* 1957).

Attempts to isolate the toxic component from the blood of DDT-prostrated cockroaches were at first hindered by difficulties encountered in the bioassay techniques. These were partially resolved in 1954 by Shankland & Kearns (1959), when they found that the concentration of toxicant present was of considerable importance, and that in fact certain concentrations could lead to doubtful or even occasionally negative bioassay responses. They confirmed the blocking of spontaneous activity in the isolated central nervous system by high concentrations of toxicant, and showed that to obtain maximum excitation of spontaneous nervous activity, dilutions of up to 80 times were sometimes required for blood samples obtained from long prostrate cockroaches.

MATERIALS.—Adult American cockroaches were obtained from a laboratory maintained colony. Both sexes were used as sources of blood, but only male cockroaches were used for electrophysiological purposes. Chemically pure *p, p'*-DDT was used. The solvents used for chromatography were distilled before use.

Collection and Processing of Blood.—During the earlier phases of this work, blood was collected by manual expression from the open ends of cut antennae and legs. This blood was frozen as it was collected to prevent subsequent clotting (Sternburg & Kearns 1952). More recently a faster and more efficient method has been used utilizing low speed centrifugation (Sternburg & Corrigan 1959). With this procedure, cells are thrown down as the blood collects, and clear serum is obtained. This serum contains the toxic principle if it is obtained from DDT-prostrate cockroaches. Shankland & Kearns (1959) showed that the active substance could be dialyzed away from the serum, when physiological saline was used as the dialysant. Their work on the relationship between concentration of toxicant and response of isolated central nerve cords was carried out with dialysates of this type. They found that physiological saline dialysates of toxic blood retained activity without significant loss after 3 hours at 25° C.

When the blood, during centrifugation, was collected in three times its anticipated volume of ethanol more active samples were obtained. Whole blood serum apparently contains an enzyme capable of destroying the toxicant, and denaturation of the protein by ethanol prevents this loss of activity. Freezing the whole blood retards but does not prevent slow loss of biological activity. At 25° C. complete loss of activity may occur in a few hours.

Blood-ethanol mixtures were therefore collected to insure maximum yields of the active substance, bagged in dialysis tubing, and dialyzed against 10 volumes of ethanol for at least 12 hours with slow agitation. The ethanol dialysates were dried under reduced pressure, and the residues were redissolved in ethanol for chromatography, or taken up in physiological saline if needed for direct bioassay without further purification. The saline was that of Pringle (1938) (NaCl 9.0 gm., KCl 0.2 gm., $CaCl_2$ 0.2 gm. per liter) buffered to pH 7 by the addition

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of 10 ml. per liter of isotonic phosphate buffer.

Bioassay Method.—A specific chemical method for detection of the toxicant has not been found. A color produced in the biologically active area of chromatograms by reaction with diazotized *p*-nitraniline (see section on chromatography) is not suitable for either qualitative or quantitative analysis of the toxicant, since the test is not specific for this substance. Many compounds present in the crude extracts react to produce similar colors. For this reason, a bioassay technique was used to detect the presence of neuroactive material. The procedure is adequately described by Shankland & Kearns (1959). Presence of the toxicant was indicated by an increase in the general spontaneous activity of an isolated central nerve cord within 1 to several minutes after application, shortly followed by spontaneous firing of giant fibers (fig. 1). In many cases, a block or partial block of spontaneous activity followed the period of excitation, and was usually maintained until the preparation was washed with fresh saline.

Chromatographic Separation of the Toxicant.—Complete isolation of the toxicant from all the components of the blood has not yet been obtained with certainty. However, a considerable degree of purification has been achieved, and comparison of the toxic substances produced by different methods has been possible. Elimination of a number of known neuroactive compounds, among them 5-hydroxytryptamine, acetylcholine, γ-aminobutyric acid and histamine, resulted from comparisons of the Rf values of these compounds with the Rf value of the toxicant in several different chromatographic systems, as well as from their behavior on the isolated central nerve cord preparation.

Both ascending and descending procedures were used. The Rf values have been slightly influenced by this variation, but adequate controls were run to make comparisons valid. The paper used was Whatman #1, previously washed exhaustively in a Soxhlet extractor with ethanol. Three different solvent systems constituted the mobile phase. To minimize bioassays, only one dimensional chromatograms were run. The solvent systems were as follows, with the figures indicating volumes: *n*-Propanol:water:formic acid = 8:1:1, *n*-Butanol:acetic acid:water = 4:1:5, Benzene:water:*n*-butanol:methanol = 1:1:1:2.

Samples to be chromatographed were dissolved in

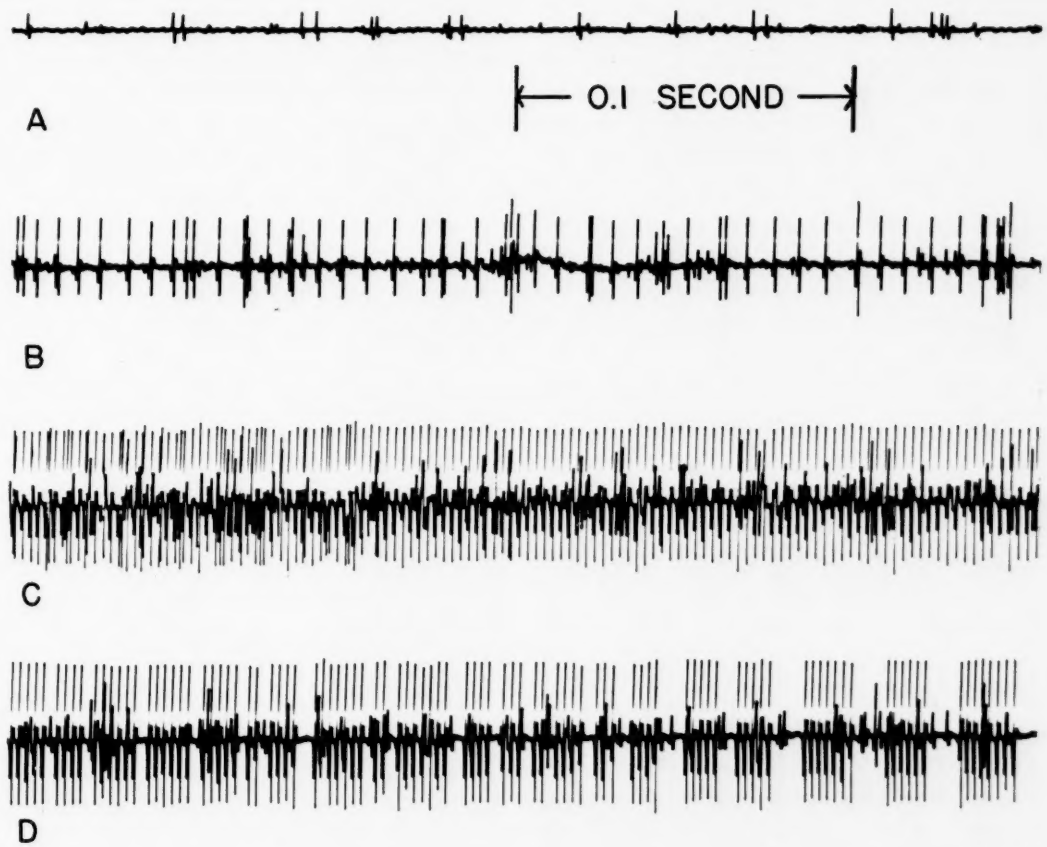

FIG. 1.—Spontaneous nervous activity recorded from the connectives between the fourth and fifth abdominal ganglia of an isolated thoracic and abdominal nerve cord obtained from an adult male American cockroach. A. Normal spontaneous activity in physiological saline. B, C, and D. Spontaneous activity 2, 4, and 6 minutes, respectively, after application of the neuroactive substance obtained from the blood of DDT-prostrated cockroaches. The neuroactive material was obtained at Rf 0.6 from a *n*-butanol; acetic acid: H^2O::4:1:5 chromatograph of the ethanol extract of the lyophilized dialysate of blood, and diluted with saline to 5% of the concentration in the original blood sample.

ethanol and applied to the paper by means of a micropipette. A stream of nitrogen was used to hasten evaporation of the ethanol when large samples were applied to the paper. With very small samples, the ethanol evaporated without assistance. The toxicant was located on chromatograms by bioassay. The developed chromatograms were sectioned, and the section either extracted with physiological saline or ethanol. If extracted with ethanol, the extracts were dried under reduced pressure, and the residue later dissolved in physiological saline for bioassay. The extract from a specific section was bioassayed by applying it to an isolated central nerve cord held at 28° C., adjusting the concentration to insure a maximum response, following the precautions found necessary by Shankland & Kearns (1959). The highest purity was obtained by carrying samples through three chromatographic separations, using each of the aforementioned solvent systems successively, and rechromatographing only the biologically active area in each system. The first system used in sequence was the propanol system. By both ascending and descending techniques the Rf of the toxicant in this system was near 0.5. Several compounds present in cockroach blood also ran to Rf 0.5 and were used to aid in the location of the toxicant without sacrificing all of it during bioassay. Tyrosine, present in cockroach blood, has a similar Rf value, demonstrated by co-chromatographing this compound with toxic blood extracts. In the propanol system either ninhydrin, diazotized *p*-nitroaniline, or diazotized sulfanilic acid were used to locate tyrosine on a strip cut from a banded chromatogram, thus locating the toxic area. In addition to amino acid markers, a weakly fluroescent band, visible in ultraviolet light, ran with the toxicant, and was also used to locate the position of the toxicant without loss of material. Treatment with alkali (NH_3 vapor, or sprays of alcoholic KOH or $NaOCH_3$) intensified the fluorescence of this area, but destroyed biological activity.

Histidine, detected on the chromatograms by its reaction with diazotized sulfanilic acid, ran with an Rf value of 0.08 and, histamine, if present, would have run with histidine. However, blood extracts chromatographed with a 3 to 1 mixture of propanol and 0.2 N NH_4OH failed to reveal the presence of histamine (Rf 0.48), whereas the presence of histidine (Rf 0.22) was confirmed.

The second system used in sequence was the butanol system. Material eluted from the active area of propanol chromatograms was spotted and rechromatographed in the butanol system. The Rf value of the toxicant was found to be 0.5 to 0.6. Tyrosine had an Rf of 0.37, and proline an Rf of 0.33. Substances that fluoresced in ultraviolet light without chemical treatment had Rf values of 0.31 to 0.42. Material that fluoresced only when made alkaline had an Rf of 0.5 to 0.6, identical to that of the biologically active area. Diazotized sulfanilic acid and *p*-nitraniline both caused color production at the Rf of the toxicant, in addition to the positive reaction with tyrosine at Rf 0.37. Epinephrine and norepinephrine, if present, would have had Rf values of 0.36 and 0.28, respectively. Methods of detection for these compounds enumerated by Block *et al* (1958) failed to reveal their presence.

Eluates or extracts of the biologically active area on butanol chromatograms were then spotted on paper and rechromatographed with the benzene, water, *n*-butanol, methanol mixture. The Rf of the toxic area was found to be 0.93. Material that fluoresced when made alkaline had an Rf value of 0.78. Diazotized sulfanilic acid and diazotized *p*-nitroaniline produced three biologically inactive purple spots, with Rf values of 0.35, 0.53, 0.71 and an orange spot at Rf 0.82. Only diazotized *p*-nitroaniline gave a pink spot at Rf 0.93, coinciding with the location of the biologically active area.

Both toxic and nontoxic blood extracts passed through the propanol and butanol systems gave similar reactions to the color tests mentioned above. In many extracts of toxic blood there seemed to be more fluorescent material than that found in nontoxic blood. Since this was not always the case its significance is unknown. Only on chromatograms developed with the benzene mixture was a difference consistently noticed. Here, chromatograms of toxic samples gave a positive pink reaction to diazotized *p*-nitroaniline at Rf 0.93. Nontoxic samples consistently failed to give any color above Rf 0.82. The areas below 0.93 were similar and no qualitative differences were noted. The positive reaction to diazotized *p*-nitroaniline in the toxic area may be due to the toxicant, but the possibility of the presence of some nontoxic substance with the same Rf does exist. The reaction is a positive test for aromatic amines, imidazols, and phenols (Feigl 1956). The iodine vapor test for nitrogenous compounds was negative and in addition the *p*-dimethylaminobenzaldehyde test and the nitric acid test for aromatic amines, and the Millon test for phenols failed to give a response. Imidazols were ruled out since diazotized sulfanilic acid is a sensitive test for that class of compounds as well as for phenols. It is possible that if larger samples were available some of the tests would prove to be positive. The exact quantity of toxicant present is not known, but it could be very small, as is characteristic of most neuroactive compounds.

Solubility of the DDT-ind ced Toxicant.—Extraction of the residue remaining after lyophilization of dialysates of toxic blood was carried out with several solvents. The insoluble portion was then redissolved in physiological saline for bioassay. The solvent extracts were subsequently dried under reduced pressure, and the residues dissolved in saline for bioassay. The toxicant was soluble in water, methanol, and ethanol, as shown by the inactivity of the insoluble portion left after extraction with these solvents. In each case, the biologically active substance was found in the solvent layer. Extraction with ethyl ether, chloroform, or carbon tetrachloride did not remove the toxic principle. Extracts made with these solvents were inactive, while the insoluble portions left behind after extraction retained all of the activity.

Attempts to extract the active principle with acetone led to the discovery that biological activity was destroyed by this solvent. Toxic dry residues extracted with acetone lost all biological activity, and the acetone extracts were also inactive. Furthermore, toxic residues when covered with a few milliliters of acetone for 5 minutes, and then brought to dryness under reduced pressure, suffered complete loss of biological activity. Acetaldehyde was found to cause a similar loss of activity, and thus it seems that the toxicant reacts with carbonyl compounds of the aldehyde and ketone class.

Stability of the DDT-induced Toxicant.—As indicated

above the biological activity was destroyed by exposure to carbonyl compounds such as acetone and acetaldehyde. Exposure to an alkaline pH also resulted in rapid inactivation. Active samples in physiological saline brought to pH 8 with NaOH for 5 minutes, and then neutralized with HCl for bioassay, were completely inactivated. Exposure of the dry material on paper chromatograms to ammonia fumes also resulted in complete inactivation. In contast, when toxic samples in physiological saline, adjusted to pH 4 with HCl and sealed in glass ampules, were held 14 hours at 80° C., no loss in biological activity was observed. Neither formic nor acetic acid inactivated the toxicant, since chromatograms developed in solvent mixtures containing these acids retained biological activity, as previously mentioned.

The toxicant was not stable when retained in whole blood or in blood serum. This may be due to enzymatic degradation. When dialyzed away from the blood or serum, and thus from the protein of the blood, the toxicant was relatively stable, whether in physiological saline, distilled water, or ethanol. Such neutral dialysates retained their activity for several days at room temperature, and for weeks at −15° to −20° C.

Removal of solvents such as water or ethanol by means of an air stream invariably resulted in complete loss of activity, but removal of solvents by a stream of nitrogen did not result in loss of activity. This suggests that the toxicant may be readily degraded by oxidation. This apparent susceptibility to oxidation has made isolation difficult due to gradual loss of activity in the many steps necessary during chromatography and extraction.

Sodium and Potassium.—Wide fluctuations in the sodium and potassium ratios are known to occur in insect haemolymph. In the cockroach, Roeder (1948) found that the spontaneous electrical activity of isolated cords continued for 25 hours in saline containing from zero to 10 mM potassium. Concentrations of 20 mM or more were required to cause immediate increases in spontaneous activity. Axonic conduction was not affected by less than 40 mM potassium, and trans-synaptic conduction remained normal in zero to 32 mM potassium. Since wide fluctuations of these ions fail to disrupt the functions of isolated nerve cords, it seemed unlikely that the neuroactivity of blood from DDT-prostrate cockroaches could be due merely to changes in ion levels. Nevertheless, cell-free blood samples from normal and DDT-prostrate cockroaches were analyzed by flame photometry for potassium and sodium. In two toxic blood samples the concentrations of potassium were 14.9 and 16.4 mM, and of sodium 142 and 152 mM. Normal blood concentrations of potassium were 13.8 and 15.2 mM, and of sodium 130 and 142 mM. These compare favorably with a potassium range of 17.3 ± 4 mM and a sodium range of 107 ± 25 mM reported by Tobias (1948) for serum from normal American cockroaches. It is apparent that no deviation from normal levels of either potassium or sodium occurred in the blood during DDT-prostration.

In Vivo Production of Toxicant by DDT.—American cockroaches of both sexes were treated topically by application of 100 μg. DDT in 10 μl. ethanol to the prothoracic sternum. The cockroaches were kept after treatment at 35° C. for about 14 hours. At this temperature and dosage no symptoms of poisoning appeared (Vinson & Kearns 1952). Transfer to 15° C. resulted in prostration of the cockroaches within 15 to 30 minutes. This procedure was followed whenever toxic blood samples were collected because it afforded exact control over the time of prostration. Prostration at 35° C. with production of toxic blood can be accomplished with higher dosages of DDT, but the time at which prostration occurs is variable, making it difficult to obtain cockroaches prostrate for definite lengths of time. Shankland & Kearns (1959) demonstrated that the level of toxicant was at a maximum approximately 3 hours after prostration and remained at this level for many hours thereafter. For this reason, for production of the toxicant for chemical and physiological purposes, a 4-hour period of prostration was arbitrarily chosen to insure a maximum titer of the substance in the blood. The cockroaches were bled at 15° C. or lower in order to prevent loss of toxicant. Ten male cockroaches yielded about 0.5 ml. of blood, enough for exploratory chromatographic work. From 5 to 25 ml. blood samples were collected for experiments involving successive chromatographic separations during attempts to isolate the active substance.

In Vitro Production of Toxicant by DDT.—Since DDT is believed primarily to affect the sensory nervous system of the intact animal, it seemed desirable to determine if treatment of nervous tissue isolated from the insect might result in toxicant production. Direct application of DDT to isolated central nervous tissue did not result in toxicant production, agreeing with the observed failure of DDT to act directly on central nerves (Roeder & Weiant 1946). However, application of DDT externally to the cerci of a system composed only of thoracic and abdominal central nervous tissue with the cercal nerves and cerci intact led to the gradual accumulation of a neurotoxic agent in the saline bathing the preparation. The procedure used was to excise the central nerve cord plus intact cerci from a normal cockroach, and place the exposed nerves in a shallow dish containing approximately 50 μl. of physiological saline. Electrodes for recording nerve potentials were attached to the cercal nerves of one cercus, and to the connectives between the fourth and fifth abdominal ganglia of the cord. Recording electrodes could also be used for electrical stimulation by changing the electrical circuit with a switch so that the wires led from a stimulator (Grass S4C) rather than to a preamplifier (Grass P-4). In some cases two pairs of electrodes were attached to the cercal nerves so that the effect of stimulation could be observed presynaptically in the cercal nerves, as well as postsynaptically in the central nerve cord. After 15 to 20 minutes observation of the preparation to verify normal behavior and response, one μl. of peanut oil saturated with DDT was applied topically to each cercus with care taken to prevent its flow into the saline. The cerci were suspended above the saline, and thus no DDT was present in the saline. Two other nerve cords with intact cerci were also placed in the same dish, and their cerci were treated with DDT at the same time. A fourth nerve cord with intact cerci was present but was not treated with DDT. Recording electrodes were also attached to this cord between the fourth and fifth abdominal ganglia, and to its cercal nerves.

Within a few minutes after application of DDT to the cerci, there was an increase in the spontaneous activity of the cercal nerves of the treated cord being monitored. The general level of activity increased many times above

normal, reaching a steady maximum in 15 to 20 minutes. Electrical stimulation of the cercal nerves resulted in a single propagated presynaptic spike at this time and throughout the entire experiment. Postsynaptically, the response was initially one for one, but gradually multiple after-discharges become apparent, and about 30 minutes after application of DDT multiple responses were consistently obtained. At this time spontaneous trains of impulses appeared in the central nerve tissue of the treated cord. In addition, spontaneous trains began to appear in the untreated cord. Its cercal nerves showed some increase in activity, but not to the extent found in the cercal nerves of the treated preparations. Eventually, both treated and untreated cords underwent prolonged high frequency after-discharges followed by a sudden depression of activity and frequently a complete block. The time for this varied in different experiments, but usually occurred between 1 and 2 hours after the start of the experiment.

The bathing saline, or perfusate, was removed and later applied to normal isolated central nerve cords, which respond in typical fashion for the presence of the toxicant. Furthermore, such perfusates, when lyophilized, extracted with ethanol, and chromatographed in the butanol system, yielded a neurotoxic area with the same Rf as the toxic material found in blood from DDT-prostrate cockroaches.

In Vivo Production of Toxicant by Electrical Stimulation.—The occurrence of trains of high frequency impulses in the sensory nerves must result in a constant bombardment of the sensory-central synapses. If the toxicant present in DDT-prostrated cockroaches is a result of this excessive sensory nerve activity and consequent bombardment of sensory-central synapses, then it seemed possible that high frequency electrical stimulation of sensory nerves might also lead to toxicant production. To test this theory, a boxlike grid was constructed consisting of alternate negative and positive wires, spaced at intervals of 3 mm., so that continual contact by the cockroaches was assured. The grid was attached to an inductorium powered by a Variac set at 6 volts. The setting of the inductorium was determined by observing the effects on the trapped cockroaches, so that they just barely showed visible signs of electrical stimulation. Under these conditions prostration eventually occurred, generally in 2 to 4 hours. The cockroaches were bled 2 to 4 hours after prostration, and the blood bioassayed on excised central nerve cords from normal cockroaches. Such blood proved to be active, causing the same type of excitation and block as that produced by blood from DDT-prostrate cockroaches.

Blood obtained from electrically prostrated cockroaches was lyophilized, the residue extracted with ethanol, and the extract chromatographed in the butanol system. Bioassays of these chromatograms indicated the presence of a neuroactive substance of Rf 0.5, identical to the Rf of the toxicant found in the blood of DDT-prostrate cockroaches.

In Vitro Production by Electrical Stimulation.—Electrical stimulation of central nerve cords resulted in the release of neuroactive material into the perfusing saline. This experiment has been replicated five times, with varying degrees of success. The results obtained from a typical experiment are discussed.

The thoracic and abdominal central nerve cord was excised from a normal male adult cockroach and placed in a trough containing 30 μl. of saline. One electrode from a Grass S-4 stimulator was attached to the connectives between the fifth and sixth abdominal ganglia. The negative stimulating electrode was submerged in the saline. Recording electrodes were attached between the second and third abdominal ganglia, and the preparation left in this position for 1.5 hours at 28° C. without stimulation. Spontaneous activity was maintained at normal levels during this period of observation.

After 1.5 hours, the cord was stimulated at a frequency of 100 stimuli per second. The duration of each stimulus was 0.1 msec., and the voltage 1.05. Stimulation was continued at this rate for 30 minutes. During the first few minutes, the cord responded to each stimulus, but after 3 minutes it began to skip, although in general it responded to most stimuli. Stimulation was stopped briefly at 10-minute intervals to permit observation of the spontaneous activity. There was a gradual reduction in the general activity and after 30 minutes the spontaneous activity was very depressed. At this time the saline was withdrawn and saved for bioassay. The stimulated cord was then washed five times during the next 5 minutes. Spontaneous activity increased gradually to a fairly normal level.

Another nerve cord, excised from a male cockroach, was placed in fresh saline and observed for 30 minutes, during which time normal spontaneous activity was maintained at a stable and constant level. The saline was then carefully withdrawn and the perfusate obtained from the stimulated preparation pipetted into the trough. Within 30 seconds the first large spikes appeared. These rapidly increased in frequency and 2 minutes after the application of the perfusate reached a maximum level, when they sounded much like a siren on the loud speaker. This extremely high activity continued for the next 5 minutes, then gradually decreased during the next 10 minutes. At this time, 17 minutes after application of the perfusate, the spontaneous activity was recorded as irregular trains of impulses, superimposed over a depressed background of smaller spikes.

Although somewhat difficult to demonstrate, electrical stimulation of the cercal nerves of an isolated cord also resulted in the production of a neuroactive substance. The thoracic and abdominal central nerve cords of two cockroaches were positioned parallel to one another in a porcelain through, and barely covered with 30 μl. of physiological saline. Recording electordes were attached to the abdominal section of each cord. One nerve cord had its cerci intact, with stimulating electrodes attached to the cercal nerves of one cercus. Postsynaptic responses of the stimulated preparation were observed and an attempt was made to stimulate at frequencies below those causing fatigue. The duration of each stimulus was 0.4 msec., with the voltage varied between 0.2 and 0.5 volts as necessary to obtain postsynaptic responses. The frequency of stimulation was varied between 1 and 10 per second, with an attempt made to maintain the highest nonfatiguing rate possible. After 1 or more hours of stimulation, the spontaneous central activity of the stimulated cord was observed to increase above normal, and there was a tendency for postsynaptic responses to be multiple. Spontaneous central nerve activity of the

stimulated preparation tended to become more and more excited, and at this time bursts of higher than normal spontaneous activity appeared in the nonstimulated cord. In two experiments, nearly 3 hours were required to excite the nonstimulated cord, although in one instance this occurred within 1 hour. The experiment has also failed on occasion. However, the experiments indicate the release of neuroactive material into the saline bathing the preparation.

DISCUSSION AND CONCLUSIONS.—Since electrical stimulation, without the use of a chemical, leads to the production of toxic material, this substance must be a naturally occurring compound, and not a metabolite of DDT. In one way, electrical stimulation may simulate certain phases of DDT-poisoning by causing an increase in the afferent impulses reaching the sensory-central synapses.

Chromatographically, the DDT-induced toxicant and the substance produced by electrical stimulation behave the same. They have similar solubility characteristics, are stable under identical conditions, and can be inactivated by the same treatments. Therefore, it is likely that they are identical compounds.

The source of the toxicant is apparently the nervous system itself, and it comes at least in part, if not entirely, from the central nervous system. Isolated thoracic and abdominal nerve cords, with their cerci intact and coated with DDT, liberate an identical substance into physiological saline. Under these conditions, the toxicant appears in the perfusing saline during and after the period of high spontaneous activity generated in the central nerve cord. This high activity in the central nerve cord arises after a period of constant bombardment of sensory-central synapses owing to the afferent impulses generated in the sensory nerves of the cerci by direct action of DDT.

Direct electrical stimulation of the abdominal region of an isolated thoracic and abdominal central nerve cord at frequencies up to 100 per second, and above the minimal voltage for giant fiber response, over a period of 20 to 30 minutes, leads to the liberation of a biologically identical substance in the saline bathing the preparation. This indicates that the central nervous system itself can produce the toxicant. Whether or not the central nervous system is the only source of the toxicant is not clear, since electrical stimulation of the cercal nerves of an isolated central nerve cord has occasionally resulted in the release of the toxicant into the perfusing saline. Its presence, in these cases has followed the initiation of great central nerve spontaneous activity, and therefore perhaps even here the toxicant originates only from central nerve tissue. It is not impossible, however, that sensory nerve tissue may have partially contributed to the total amount released.

The biological activity of the toxicant is manifested by a great increase in the spontaneous activity of the central nerve cord. This eventually becomes cyclic and consists of trains of rhythmic impulses, often persisting for several minutes (fig. 1). The individual spikes are all alike, and have a potential considerably greater than the normal potential of a single giant fiber spike, suggesting summation and synchronization of firing of more than one fiber. In addition to disrupting spontaneous activity, the toxicant causes facilitation and block of transsynaptic impulses (Shankland & Kearns 1959).

The neuroactivity of the toxicant and its liberation during central nervous system activity, imply that it has some role in neurotransmission. Confirmation of this possibility necessarily must await chemical identification of the substance. Although the structure of the toxicant is unknown, it is definitely distinct from other known or suspected neurohumoral agents. Chromatographic and biological behavior have eliminated from further consideration such compounds as epinephrine, norephinephrine, histamine, γ-aminobutyric acid, γ-butyrobetaine, 5-hydroxytryptamine, acetylcholine, and excess potassium.

In recent years numerous reports have appeared relative to biologically active substances present in the blood of insects, following various treatments either with chemicals or by some form of stimulation or restraint. Beament (1958) found that mechanically immobilized cockroaches were affected by a form of paralysis, and that a similar physiological state could be produced by electrical or mechanical stimulation. By means of parabiotic and injection techniques he found evidence of a pharmacologically active substance in the blood of mechanically restrained cockroaches. Mechanically stimulated cockroaches showed the same sort of paralysis, persisting for days, as the paralysis of mechanically restrained cockroaches. On the other hand, he found that electrically stimulated cockroaches soon recovered from prostration and did not subsequently become paralyzed. Because of differences in the methods used by Beament and the techniques reported in this paper, the validity of comparisons is uncertain. However, the slow and persisting paralysis due to mechanical restraint or stimulation is not characteristic of the DDT-induced toxicant. The temporary paralysis resulting from electrical stimulation is a characteristic of injected DDT-induced toxicant, however, and it is likely that it is distinct from the mechanically induced material.

Ozbas & Hodgson (1958) made extracts of whole corpora cardiaca of the American cockroach. They found that such extracts caused, after 5 minutes, a decrease in the frequency of spontaneous nervous activity in an isolated cockroach central nerve cord, and when injected into cockroaches caused decreased and stereotyped locomotor behavior. Extracts made from central nerve cords did not cause a similar depression, nor did they cause a disruption of the normal locomotor behavior when injected into cockroaches. They cite surgical and histological evidence indicating that the active principle in the corpora cardiaca originates in the brain. Since the brain and the corpora cardiaca were not present in the experiments reported in this paper involving isolated thoracic and abdominal nerve cords, the substance reported by Ozbas and Hodgson appears to be distinct from the DDT-induced toxicant, unless the agent from the corpora cardiaca can also be synthesized by central nerve tissue during excessive nervous activity.

Colhoun (1958) reported that blood from cockroaches prostrated by either DDT or tetraethyl pyrophosphate caused an increase in the spontaneous activity of isolated nerve cords. He suggested that there might be a common factor present, but because of the presence of TEPP in the blood of TEPP prostrated cockroaches, could not with certainty demonstrate this. Colhoun found that blood from DDT-prostrate cockroaches contained ab-

normally high amounts of corpora cardiaca hormone, indicating that some of the activity of whole blood may be due to this substance. He states further, however, that there are high titers of other active substances in such blood, one of them arising from the corpora allata. Since neither corpora allata nor corpora cardiaca are required for the production by isolated nerve cords of the toxicant here demonstrated, the toxicant is distinct from the secretions of these glands, but it is possible that the toxicant is one of the other unknown substances that Colhoun has found.

In addition to the release of the toxicant owing to stimulation by DDT action or by electrical means, an apparently identical substance is released during the excitation that follows the application of 10^{-3}M TEPP to an isolated thoracic and abdominal central nerve cord (Sternburg *et al.* 1959). Again, the corpora cardiaca are not necessary for its production. The release of toxicant apparently occurs during the burst of extremely high spontaneous activity resulting from facilitation of synaptic transmission as cholinesterase is inhibited by the direct action of TEPP. The toxicant accumulates in the saline bathing the isolated nerve cord in amounts sufficient to completely block all spontaneous activity. That the toxicant rather than TEPP is responsible for this block is shown by the fact that the spontaneous activity may be restored to a normal level by washing the preparation with fresh 10^{-3} TEPP. The restored spontaneous activity is maintained at normal levels for many hours, in spite of the presence of high concentrations of TEPP and consequent complete inhibition of cholinesterase. Reapplication of the initial TEPP perfusate causes the nerve cord to become excited again and eventually depressed or blocked. These events support the suggestion that the toxicant may be involved in neurotransmission within the central nervous system.

The release of the toxicant by the action of both DDT and TEPP suggests there must be a common factor in their modes of action. Although DDT itself does not inhibit cholinesterase, Sternburg *et al.* (1959) have shown that *in vivo* the cholinesterase of DDT-prostrate cockroaches is in an abnormal state, in that DDT-prostrate cockroaches are protected against the action of a short-lived but irreversible cholinesterase inhibitor, tetramethyl pyrophosphate (TMPP), if they are kept in a DDT-prostrate condition until the excess phosphate has hydrolyzed. This observation implies that some substance is present that prevents TMPP from reaching an active site on the enzyme. The effect could be due to a reversible inhibition of cholinesterase during DDT-prostration or to a change in membrane permeability, isolating cholinesterase. An action of this sort would account for the initiation of excessive central nervous system activity and the resultant release of neuroactive material.

The nature of the initial action of DDT on sensory nerves remains obscure. The importance of para substitutions on the phenyl rings, and the necessity for definite ethane substitutions suggest that DDT must fit on an active site of some constituent of either the sensory nerve endings or associated structures. In so doing, instability of afferent activity is initiated, leading eventually to hyperactive synaptic transmission and consequent excessive central nervous activity. During excessive central nervous activity a neuroactive substance is released into the blood in abnormally great amounts, which disrupts normal nerve functions. Thus the final action in a series of events initiated by DDT may occur at sites far remote from the original site of application.

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The Supposed Correlation between the Ratio X_5 and DDT-Resistance in House Flies[1,2]

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ABSTRACT

The ratio of X_5, defined by Bigelow and LeRoux as the width of the second abdominal sternite divided by its length, was measured on both sexes of eight strains of house flies, *Musca domestica* L., differing in their susceptibility to DDT. Resistance levels were determined by topical application of the insecticide followed by a maximum likelihood probit analysis of the results. Significant differences among strains and among rearing jars within strains were found, as in an earlier study by Sokal and Hunter. Significant differences between resistant and nonresistant strains were found for X_5, but the difference was opposed to that reported by Bigelow and LeRoux and by Morrison. Further analyses led to the conclusion that the difference between resistant and nonresistant strains is due to the particular strains composing the present study and that no correlation between resistance and X_5 can be demonstrated.

This paper is one of a series reporting the findings of a research project on the genetics and ethology of DDT-resistance in *Drosophila* and house flies, *Musca domestica* L., conducted at the Department of Entomology of the University of Kansas. It reports a new attempt to correlate the resistance status of eight laboratory strains of house flies with the ratio of the width of the second abdominal sternite to its length.

Since the discovery of insecticide resistance in house flies and other insects, investigators have been looking for morphological correlates of the phenomenon. Such investigations have been made with at least two motives: research workers hope to be able to find a morphological basis for the resistance mechanism and therefore anticipate thicker or longer appendages, modified cuticle *et cetera*. Other workers are looking for (genetic) morphological correlates of resistance, which would enable them to determine the tolerance of a given individual without having to subject it to a toxicological assay.

Geneticists will probably agree that, if the phenomenon of insecticide resistance in different strains of the same species is based on different physiological mechanisms, no overall or constant morphological correlate is likely to be observed. Actually, it might be stated that only in those cases where the genetic mechanism in the strains is identical and is also simple, based on no more than one or two genes, would we expect constant morphological correlates from the pleiotropic action of the genes determining resistance.

Many correlated characters of a morphological and physiological nature and that of behavior have been reported in resistant strains of various insects. Crow (1957) has pointed out that these are probably of " . . . no significance, being simply properties of the particular strain studied and not in general associated with resistance."

Several correlations with resistance have been reported for the house fly. These correlations are reviewed by Sokal & Hunter (1955). None seems to have been confirmed by later workers. A character that has received more than the average amount of attention was first studied by Bigelow & LeRoux (1954), who computed the ratio of the maximum width of the second abdominal sternite of the house fly over the maximum length of the same sclerite. This ratio, which they called X_5, was appreciably greater in the three resistant strains than in six susceptible ones. It should be stressed that Bigelow and LeRoux did not measure the resistance status of their flies; they labeled a strain resistant or susceptible according to reports in the literature.

Subsequent work by Sokal & Hunter (1955) showed that reportedly or "nominally" resistant strains were not necessarily resistant in fact. It follows, therefore, that the actual resistance must be determined by insecticide assays at or near the time of measurement. Sokal & Hunter (1955) measured 14 morphological characters in nine house fly strains and were unable to show consistent correlation between resistance and any morphological character or the ratios reported by Bigelow and LeRoux. They found appreciable differences among rearing jars and from generation to generation, which were ascribed to differences in the microclimate or microflora of the rearing vessels.

The question was reopened by Morrison (1957) who measured males and females of 19 different laboratory strains. Morrison concluded that the " . . . ratio X_5 varied from culture to culture but in general large ratios were associated with cultures *said to be DDT-resistant* and small ratios with cultures *said to be susceptible, but the correlation was not perfect.*" (Our italics.) He also found that "variation between samples drawn from the same cultures with long time intervals were significant in one instance but not in another." As will be shown herein the significance of some of Morrison's findings is questionable. After the publication of Morrison's paper we decided to check our earlier conclusions regarding the absence of correlation between the ratio X_5 and DDT-resistance. To this end a series of measurements was undertaken, which will be included in this report.

RE-ANALYSIS OF MORRISON'S DATA.—Before proceeding with an account of our own work we shall examine in some detail Morrison's (1957) findings. In table 1 of his paper Morrison lists mean values of the ratio X_5 for 22 different sets of measurements of 19 laboratory cultures. Ratios are given both for males and females accompanied by the initials of the person measuring the flies and by the reported susceptibility status of the strain. We arrayed

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these data by magnitude of the X_5 ratio and segregated them into a susceptible and a resistant group by their reported resistance status. Wilcoxon's ranking test (Bliss & Calhoun 1954, p. 88) was then performed to show whether a difference with respect to X_5 existed between the resistant and susceptible groups. Another test that was performed on these same data was the Mann-Whitney *U* test (Siegel 1956). Both tests gave consistent results. No correlation whatsoever could be found between DDT-resistance and X_5 in the females. By the Wilcoxon test the difference between expected and observed values was only 0.53 standard deviations, while the *U* value of the Mann-Whitney test was 33 (maximal critical 5% value =19). When we examined the data for the males we did find association. The Wilcoxon test yielded a difference 2.26 times the standard error ($.05>P>.02$) and the Mann-Whitney *U* value was 14.5 (maximal critical value =21 at 5% and 13 at 1%). We must therefore conclude that association between resistance status and X_5 level was present only in the males.

However, we cannot be sure that such an association actually does exist, since the resistance status of the flies at or near the time of measurement was not investigated. A further complication in accepting Morrison's interpretation is the fact that resistance status is usually investigated in females. The only significant association is therefore between male morphology and female resistance levels.

MATERIALS AND METHODS.—The work reported in this paper is based on eight strains of house flies that have been maintained for some time in the Housefly Laboratory of the Entomology Department at the University of Kansas. These strains are the following:

BELL^{---} The Bellflower strain obtained from the Citrus Experiment Station, Riverside, California in 1953.

D^{+++} A strain identified with that symbol obtained from the University of Illinois, Urbana in 1957.

ILL^{---} The Illinois Field Strain, obtained from Urbana in 1957.

LC^{---} Lab California, a nonresistant strain obtained from Riverside in 1953.

LDD^{---} A lindane-, dieldrin- and DDT-resistant strain obtained from the U.S.D.A. Laboratory at Orlando, Florida, in 1953.

ND^{---} The same strain as NH, but received from Riverside in 1957.

NH^{---} The 1948 NAIDM nonresistant strain obtained from a subculture at Kansas State College in 1953.

OL^{---} The Orlando #1 strain, DDT-resistant, obtained from Orlando in 1957.

These strains are kept under standard conditions of culture in our laboratory of the Department of Entomology of the University of Kansas. They are maintained in large numbers in a population cage kept in a constant-temperature room at 80° F. Flies are given fresh food each day by placing a small amount of cellucotton in a Dixie food dish with a solution of powdered milk added. Oviposition takes place on special cups containing Chemical Specialties Manufacturers' Association (CSMA) fly medium which is prepared by using 1,000 grams of dry CSMA meal, 3,000 c.c. water, 50 grams of brewers' yeast, 100 c.c. Karo syrup, and 113 grams of bakers' yeast. Eggs from these oviposition cups are emptied into quart jars of this medium. The jars are kept in the same temperature room with the adult flies. Flies are permitted to emerge in the jars and are subsequently placed in new stock cages. These stock strains have not been selected for resistance under the pressure of DDT or other insecticides during the time they have been maintained in this laboratory.

Flies for the morphological measurements were reared from approximately 100 eggs picked at random from many thoroughly mixed eggs collected from a population cage and placed in a half-pint milk bottle, two-thirds full of CSMA medium. The adults were permitted to emerge in the culture bottles, fed with milk, and were removed for dry storage 5 days after emergence of the first adults.

Females from three separate rearing jars were taken from strain BELL, two jars each from strains D^{+++}, ILL LC, LDD and OL and one jar each from ND and NH. Males were taken from two jars for each strain.

The morphological characters measured were those necessary to obtain the X_5 ratio: X_1, the maximum width of the second abdominal sternite, and X_2, the maximum length of the same sclerite. X_5 was computed by dividing X_1 by X_2. Since the second abdominal sternite was already being examined under the microscope, it was decided to count the number of setae thereon. We had not previously taken this measurement, which we shall call Q, but it is serially homologous to the previous characters O and P (Sokal & Hunter 1955), which are the number of setae on the third and fourth abdominal sternite, respectively. The measurements were carried out with a Leitz Ultrapack microscope, using a No. 11 objective and a Spencer AO movable eyepiece micrometer. All measurements were carried out by Mr. William L. Peters.

At approximately the same time the flies for measurement were obtained we also undertook a series of DDT-resistance assays of our various strains in connection with a different research project. These resistance assays enabled us to correlate our morphological findings with the actual resistance status of the flies. While we are unable to correlate morphology with DDT-resistance in the very same generation, we have readings sufficiently close to the measured generation so that considerable reliance can be placed upon our DDT-assay readings. Flies that were to be assayed were reared from approximately 2,000 eggs collected from a population cage and placed into a standard No. 10 tin can lined with a plastic sack. The can contained CSMA medium to a height of 10 cms. Two days before the first emergence the pupae were collected by flotation and placed in a 1-pint ice cream carton, 150 to 200 pupae per box.

Adults from 4 to 6 days old were tested. Between emergence and testing they fed from a lump of sugar laid on the wire screen covering the ice cream carton, as well as a vial containing milk. The flies were anesthetized by CO_2 for no more than 7 minutes during treatment. They received DDT in acetone solution by topical application on the thorax. The toxicant was administered with a ratchet mechanism based on a design by Dr. W. N. Bruce of the University of Illinois. The liquid issued from a 0.25-c.c. tuberculin syringe in a drop size averaging 2.15 microliters. Treated flies were placed in a paper box and given sugar and milk. Mortality counts were taken 24 hours after treatment. The treatment room was maintained at 80° F.

RESULTS.—The results of the DDT-assays are shown

Table 1.—Results of repeated resistance assays of seven strains of house flies.

STRAIN	SEX	DATE	LD_{50} IN μg. DDT PER FLY
OL	♀	Dec. 16, 1957	74.08
	♀	Dec. 27, 1957	77.75
	♀	May 16, 1958	61.43
	♂	Dec. 16, 1957	3.96
	♂	Dec. 24, 1957	1.17
ILL	♀	Jan. 4, 1958	25.36
	♀	May 16, 1958	4.48
D^{+++}	♀	Dec. 17, 1957	19.02
	♀	Dec. 20, 1957	.493
	♀	Dec. 27, 1957	2.18
	♀	Jan. 6, 1958	1.83
	♀	May 16, 1958	.306
ND	♀	Dec. 18, 1957	0.257
	♀	Dec. 27, 1957	.172
	♀	May 16, 1958	1.00
LDD	♀	Jan. 6, 1958	0.0726
	♀	May 16, 1958	.580
BELL	♀	May 16, 1958	0.293
NH	♀	Nov. 18, 1957	0.169
	♀	Dec. 17, 1957	.0569
	♀	Jan. 6, 1958	.162

in table 1. Only 21 out of 45 assays are listed in this table since it was found that a number of the assays exhibited to much heterogeneity of response that it was impossible so fit a regression slope to them. If this appears to be an unusually large proportion of unusable data, we would point out that the LD_{50}'s given in table 1 are maximum likelihood estimates computed by means of a digital computer program developed by Sokal (1958), who has pointed out elsewhere that many of the DDT-resistance assays published in the literature based on eyefit curves are probably not valid (Sokal 1959).

On the basis of the findings in table 1, the following array of resistances can be established from most to least resistant: 1, OL; 2, ILL; 3, D^{+++}; 4, ND; 5, LDD; 6, BELL; 7, NH.

Through an oversight assays for strain LC were unavailable. Previous work (Sokal & Hunter 1955) showed that LC was a typical nonresistant strain and it was therefore given the same resistance level here as the ND strain. For purposes of the analysis of variance reported below strains 1 to 3 were considered resistant, while strains 3 to 7 plus LC were considered nonresistant. It should be noted that the LDD strain had lost resistance when compared with its previous status (Sokal & Hunter 1955). We cannot be entirely sure of the order of resistance of strains 4 to 7, above. However, several tentative rearrangements failed to yield appreciably different results in the subsequent analyses.

Table 2 shows the means and standard errors of the various measurements for both sexes and all strains. The data were subjected to analyses of variance to determine (1) whether significant differences occurred among the eight strains of the study, (2) whether differences existed among rearing jars within a strain, (3) whether the character means differed between the resistant and the nonresistant strains, and (4) whether differences in means took place among the resistant strains. The four lines in table 3 correspond to the above-mentioned four tests.

Table 2.—Means, standard errors, and sample sizes for four characters in both sexes of eight strains of house flies.[a]

STRAIN AND SEX	SAMPLE SIZE	CHARACTER							
		X_1		X_2		X_5		Q	
		$\bar{X}$	$\pm s_{\bar{x}}$	$\bar{X}$	$\pm s_{\bar{x}}$	$\bar{X}$	$\pm s_{\bar{x}}$	$\bar{X}$	$\pm s_{\bar{x}}$
OL♀	25[b]	0.504	0.0050	0.646	0.0076	0.783	0.0096	68.7	1.74
OL♂	25	.466	.0085	.590	.0090	.790	.0144	74.1	1.62
ILL♀	25	.576	.0093	.585	.0072	.984	.0101	76.0	1.00
ILL♂	25	.547	.0077	.589	.0057	.930	.0150	75.2	1.58
D^{+++}♀	25	.617	.0073	.677	.0092	.914	.0148	72.8	1.31
D^{+++}♂	25	.597	.0088	.617	.0084	.968	.0116	77.2	1.42
ND♀	10	.582	.0121	.658	.0089	.884	.0199	76.7	2.18
ND♂	25	.558	.0071	.564	.0045	.992	.0144	82.9	1.05
LC♀	24	.662	.0090	.667	.0063	.993	.0132	90.1	1.85
LC♂	—	—	—	—	—	—	—	—	—
LDD♀	25	.552	.0061	.576	.0075	.961	.0095	72.0	1.43
LDD♂	25	.577	.0067	.578	.0040	1.000	.0136	78.5	1.89
BELL♀	25	.570	.0089	.634	.0104	.901	.0120	80.2	1.92
BELL♂	25	.612	.0117	.627	.0029	.975	.0175	85.2	1.12
NH♀	25	.612	.0101	.654	.0089	.937	.0149	75.8	1.68
NH♂	25	.577	.0093	.597	.0060	.968	.0159	78.7	1.62

[a] All linear measurements in millimeters.
[b] Only 24 females measured for character Q in strain OL.

Table 3.—Levels of significance for F-ratios based on analyses of variance of the four characters for both sexes of eight strains of house flies.[a]

	Character							
	X_1		X_2		X_5		Q	
Source of Variation	♀	♂	♀	♂	♀	♂	♀	♂
Among strains	**	**	*	n.s.	**	**	n.s.	**
Among jars within strains	n.s.	**	**	**	**	n.s.	**	n.s.
Resistant vs. non-resistant[b]	R<N**	R<N**	n.s.	n.s.	R<N*	R<N**	n.s.	R<N**
Among resistant strains	**	**	**	n.s.	**	**	n.s.	n.s.

[a] Symbols: **=P≤.01; *=.01<P≤.05; n.s.=P>.05.
[b] In this line, R<N implies that the mean for the nonresistant strains is larger than the mean for the resistant strains at the significance level given by the asterisks following N.

An examination of table 3 reveals that significant strain differences were present for every character except X_2 in the males and Q in the females, and that most of the characters also showed an added variance component among the jar means within a strain. These findings corroborate the earlier ones by Sokal & Hunter (1955). When we examine the differences between resistant and nonresistant flies we find that such differences exist for characters X_1 and X_5 and for Q in the males. Focusing our attention on X_5, Bigelow and LeRoux's ratio, we note that the nonresistant strains have significantly higher means than the resistant strains, as show by data given in tables 2 and 3. The relationships, therefore, are entirely opposite to those reported by Bigelow and LeRoux (1954) and Morrison (1957).

The conclusion may be reached that, while apparently the differences we found were opposed to those observed by other authors, DDT-resistant flies do appear to differ morphologically from the nonresistant ones. However, a closer examination of the data invalidates even this conclusion. The three resistant strains include two with a relatively high X_5 value, namely D^{+++} and ILL, while the OL strain has an unusually low X_5 ratio. When the three strains are considered together the average X_5 value is lower than the average of the nonresistant strains. It appears, however, that the actual mean value of the nonresistant strains and its significant difference from that of the resistant ones is entirely a function of the particular strains selected. These conclusions are borne out by the highly significant differences among resistant strains as shown in the fourth line of table 3.

Were resistance and X_5 correlated continuous variables we should expect different levels of the ratio to be related to different levels of resistance. This assumption was tested by correlating resistance rank and the rank of the X_5 means. The Spearman rank correlation coefficient (Siegel 1956) for these two variables is not significant for both sexes, being, however, considerably higher for the males (−0.535) than it is for the females (−0.214). In view of the fluctuations reported earlier by Sokal & Hunter (1955), the other morphological differences that can be found in tables 2 and 3 do not appear to be of much interest.

DISCUSSION.—The data analyzed in this study do not support the idea of correlation between DDT-resistance as determined by our assays and the X_5 ratio (or any of the other three measurements taken in the present study). We cannot even be sure whether a given strain will retain a relatively constant X_5 value over a number of generations. In order to investigate the latter question thoroughly, considerable work would have to be done, studying numerous strains under both constant and fluctuating environments and under very carefully controlled conditions. Some evidence has been reported elsewhere (Sokal 1959a) that the X_5 character is more stable than some of the other house fly characters, and that this stability may be related to maintenance of proper body proportions. However, in view of the very dubious proof for a correlation between X_5 and DDT-resistance and the considerable body of evidence that has accumulated against such a correlation, it seems useless to pursue this matter further at this time.

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Ethylene Dibromide: Methyl Bromide Mixtures as Fumigants against the Confused Flour Beetle[1]

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ABSTRACT

Five different mixtures of ethylene dibromide and methyl bromide were studied at three dosage levels, 8, 10, and 12 mg./l., for effectiveness as fumigants against different life stages of the confused flour beetle, *Tribolium confusum* Duv., including adults, larvae, and pupae. Eggs were included in early experiments but were omitted in later ones, since at the dosages studied, 100% mortality of this stage was characteristic for all treatments. The insects were exposed to fumigant for a 5-hour period, and mortality counts were made at 1, 2, and 14 days following completion of exposure. The effect of fumigation on larval emergence from eggs laid by adults surviving treatment was also studied.

Most of the mixtures investigated were more effective against all life stages of the insect studied than was methyl bromide alone. In addition, many of the mixtures were more effective than either ethylene dibromide or methyl bromide, on the basis of mortality 1 day following exposure to the fumigant. Thus, insect kill is more rapid with mixtures than with either component alone.

Larvae emerged from eggs laid by adults surviving treatment at all dosage levels of methyl bromide, and of mixtures containing less than 1.0 mg./l. of ethylene dibromide. No larval emergence was observed from eggs of adult beetles surviving treatments containing more than 1.0 mg./l. of ethylene dibromide.

Advantages of the mixtures for p' ctical fumigation are apparent from results of this study.

Fumigation with a volatile toxicant is often the most convenient and effective method for controlling insects in stored commodities, in storage buildings, and in the soil. A wide choice of fumigants is available for these applications, including single toxicants and mixtures of two or more components. Among the fumigants that have been in general use for a number of years are methyl bromide (CH_3Br) and ethylene dibromide (CH_2BrCH_2Br).

Fisk & Shepard (1938) reviewed the early use of methyl bromide as a fumigant, and gave experimental data on the toxicity of this compound to four species of insects. Lindgren *et al.* (1954) included both ethylene dibromide and methyl bromide among the 10 fumigants tested against eight species of stored-product insects. Krohne & Lindgren (1958) also included both toxicants in a study of the susceptibility of life stages of the rice weevil, *Sitophilus oryza* (L.) to seven different fumigants.

Despite the proven effectiveness of both ethylene dibromide and methyl bromide as fumigants, no experimental work has been reported on the fumigant activity of mixtures of the two, although fumigant mixtures are commonly used in practical pest control, and are frequently more effective than single toxicants. Earlier work in this laboratory (unpublished) indicated that ethylene dibromide/methyl bromide mixtures are more effective against adults of the confused flour beetle, *Tribolium confusum* Duv., than either toxicant alone, when compared at equal dosage rates. In this study the toxicity of the mixtures and the components was determined only against the adult stage of the confused flour beetle. The investigation reported here was undertaken for the purpose of determining the relative toxicity of ethylene dibromide, methyl bromide, and mixtures thereof, to all of the life stages of *T. confusum*.

MATERIALS AND METHODS.—The insects[3] used in this study were reared on a mixture of whole wheat flour and white corn meal (50:50 by weight), using that portion of the mixture that would pass an 18-mesh sieve. New cultures were established on this media at weekly intervals by transferring adults from a stock culture to fresh media. The cultures were reared in one-quart fruit jars, in a room maintained at 80° F.

All life stages, except eggs, were collected from such standard cultures, of known age. Eggs were collected from extra-fine white flour by sieving, following a 2-day habitation on the flour by a group of adult beetles. The different life stages used were as follows: adults, 1 to 2 weeks after emergence; larvae, third and fourth instar; pupae, quiescent, middle pupal stage; eggs, 1 to 2 days old.

The fumigants used were obtained from commercial sources.[4]

The experiments were carried out in 5-gallon (19.5-liter) glass carboys, closed with tight-fitting rubber stoppers coated with an inert epoxy resin. The apparatus used for filling the chambers is shown in figures 1 and 2.

Figure 1 shows the specially designed weighing vessels used for measuring and introducing ethylene dibromide. The fumigant was pipetted onto a plug of glass wool in the weighing vessel, and weighed on an analytical balance. Loss of ethylene dibromide from the vessel was negligible during weighing and transfer to the filling device. The vessel was then inserted into the filling apparatus at C, figure 2, and the ethylene dibromide swept into the partially evacuated fumigation chamber with the incurrent stream of air as the chamber was returned to atmospheric pressure. To ensure conversion of ethylene dibromide from liquid to gas, the bulb of the weighing vessel was warmed with steam during the filling process.

Methyl bromide was measured volumetrically in the gas burette shown as a part of the apparatus in figure 2, and introduced into the partially evacuated chamber by

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[3] Culture of *T. confusum* secured from Dr. Frank Hazard, Wilmington College, Wilmington, Ohio.

[4] Ethylene dibromide (CH_2BrCH_2Br) Westvaco Chemicals, Westvaco Chlor-Alkali Division, Food Machinery and Chemical Corporation, South Charleston, West Virginia; methyl bromide (CH_3Br) Michigan Chemical Company, St. Louis, Michigan. (Supplied by special order in a pressure cylinder without air padding.)

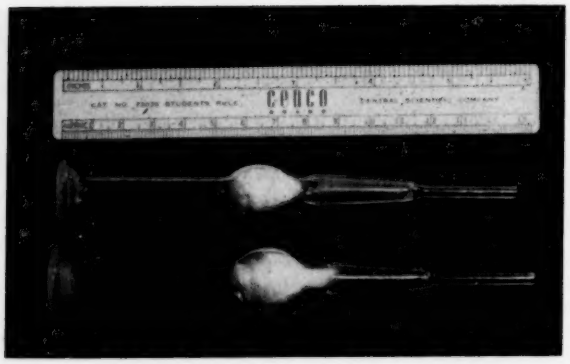

FIG. 1.—Weighing vessels used for measuring and introducing ethylene dibromide; the loaded vessel is inserted in the filling apparatus (at "C," fig. 2) for transfer of fumigant to exposure chamber.

displacement, as the chamber was returned to atmospheric pressure. Gas volumes of methyl bromide were corrected for temperature and barometric pressure prevailing at the time of filling.

When mixtures of the two fumigants were used, filling was done in the same manner as described for ethylene dibromide and methyl bromide, respectively, the two materials being introduced simultaneously. After introduction of fumigants, the carboys were placed in a constant-temperature room maintained at $74 \pm 1°$ F.

After the filled carboy had been conditioned for 1 hour in the constant-temperature room, the closing stopper was removed, the cages of insects quickly lowered into the chamber, and the carboy immediately re-stoppered. The chambers were allowed to remain in the constant-temperature room during the exposure period. In all of the experiments reported here, the exposure period was 5 hours.

Simultaneous exposure of different life stages was accomplished by the use of small cylindrical copper screen cages (2 cm.×2 cm.) fitted with screen bottoms, so designed that up to four cages could be stacked and lowered into the fumigation chamber as a unit. The cages are shown in figure 3. The cage design provides for free access of fumigant to the insects, and permits separate confinement of insects of each life stage. The cages were suspended near the center of the fumigation chamber on a wire attached to the coated rubber stopper sealing the chamber.

Upon completion of the exposure, the cages were removed and the insects from each cage were transferred onto whole wheat flour contained in a shell vial closed with a perforated plastic stopper. Insects were held in the vials, in a constant-temperature, constant-humidity room at $74 \pm 1°$ F. and $60 \pm 1\%$ relative humidity for 2 weeks, except for short intervals when they were taken to the laboratory for mortality counts.

Each experiment included exposure to each of the two fumigants alone, and five different mixtures thereof, all at a given dosage rate calculated as milligrams of fumigant per liter of space. Two groups of 50 insects each, in separate cages, were exposed for each life stage in each

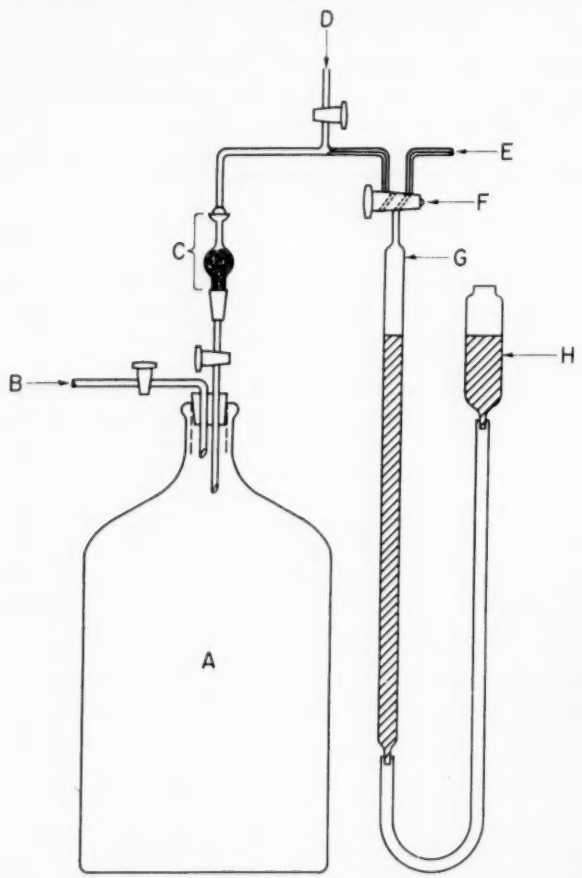

FIG. 2.—Schematic diagram of apparatus used for measuring methyl bromide, and for introduction of methyl bromide and/or ethylene dibromide into exposure chamber. (A) 5-gallon glass carboy. (B) Outlet to vacuum line. (C) Glass weighing vessel (see fig. 1). (D) Air intake used to return fumigation chamber to atmospheric pressure. (E) Inlet from methyl bromide tank. (F) Two-way stopcock. (G) Gas burette. (H) Leveling flask filled with saturated aqueous solution of $CaCl_2$.

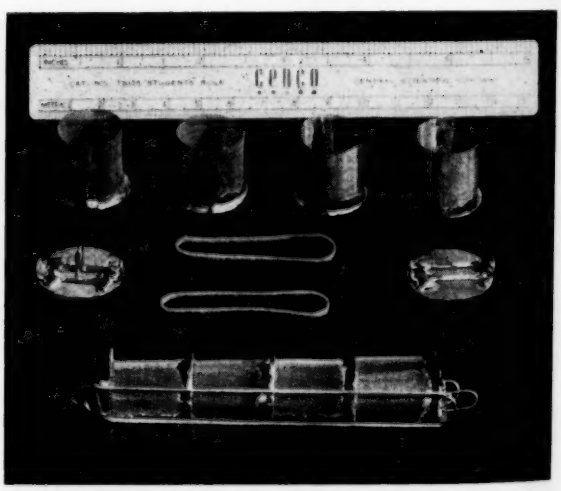

FIG. 3.—Exposure cages, and assembled stack of cages, used for simultaneous exposure of different life stages.

fumigant, in any given experiment. Each experiment was replicated five times.

Mortality counts were made on adults and larvae 1, 2, and 14 days following exposure, and on pupae and eggs 14 days following exposure. Adults and larvae were judged dead when no movement could be detected on examination under a strong light for a few minutes. For the other two stages, transformation to the next life stage (eggs to larvae, or pupae to adults) was determined 14 days after exposure. Eggs or pupae failing to transform to the next stage, as well as dead insects in the advanced stage, were considered killed by the treatment.

All mortality counts were converted to per cent mortality, corrected for mortality in the controls. In most experiments, 100% of the control insects survived to completion of the test except for eggs, where a hatch of about 80% was characteristic. The data were treated statistically by analysis of variance, and the least significant difference (L.S.D.) values calculated at the 1% and 5% probability levels.

RESULTS.—Table 1 gives the mortality data for three life stages of *T. confusum*, after a 5-hour fumigation with ethylene dibromide, methyl bromide, and five mixtures thereof, at dosage levels of 8, 10, and 12 mg./l. In the first few experiments conducted, eggs were also included. However, it soon became apparent that even the lowest dosage level, 8 mg./l., of either fumigant, or of any mixture, gave 100% kill of the eggs. This stage was therefore omitted in subsequent experiments.

From the 1-day mortality data in table 1, it is evident that the fumigant action of mixtures of ethylene dibromide and methyl bromide was in many instances more rapid than that of either fumigant alone, especially at the higher dosage levels. (See per cent mortality values marked with superscript (a) in table 1.) Thus, at dosage levels of 10 and 12 mg./l., nearly all mixtures gave significantly higher kills of adult beetles, 1 day after exposure, than either toxicant alone. Likewise, at the 12 mg./l. level all mixtures had killed a significantly higher percentage of larvae, 1 day after exposure, than either fumigant alone. There was, however, no significant difference between mixtures with respect to per cent mortality 1 day after exposure, at either 10 or 12 mg./l. for adults or 10 mg./l. for larvae. The mixtures, ranging from 10% ethylene dibromide/90% methyl bromide to 60% ethylene dibromide/40% methyl bromide, were

Table 1.—Average per cent mortality of three life stages of *Tribolium confusum*, after 5-hour exposure to different dosages of ethylene dibromide (EDB), methyl bromide (MB), and mixtures thereof.

Composition of Fumigant (%)		Cumulative Per Cent Mortality at Indicated Days After Exposure (Total Fumigant Dosage Expressed as Mg./Liter)								
		1 Day			2 Days			14 Days		
EDB	MB	8	10	12	8	10	12	8	10	12
					Adults					
0	100	0.0	19.1	44.2	0.2	21.9	52.4	4.8	42.5	68.9
10	90	0.8	43.8[a]	92.3[a]	2.6	79.3[b]	99.4[b]	33.5[b]	97.6[b]	99.8[b]
20	80	4.0	52.7[a]	97.0[a]	35.1[b]	90.1[b]	99.8[b]	97.1[b]	100.0[b]	100.0[b]
30	70	9.1	61.8[a]	97.0[a]	69.1[b]	99.2[b]	100.0[b]	99.8[b]	100.0[b]	100.0[b]
40	60	20.3[b]	61.0[a]	94.3[a]	87.3[b]	99.4[b]	99.8[b]	100.0[b]	100.0[b]	100.0[b]
60	40	34.4[b]	54.3[a]	94.6[a]	95.6[b]	99.6[b]	99.8[b]	100.0[b]	100.0[b]	100.0[b]
100	0	38.0	19.6	42.1	93.2	92.2	95.1	100.0	100.0	100.0
L.S.D. at 5% level		11.3	22.8	17.8	13.5	13.4	16.9	5.9	11.4	13.4
at 1% level		15.3	30.9	24.1	18.4	18.1	22.9	9.0	15.4	18.1
					Larvae					
0	100	6.2	59.1	75.8	8.6	64.0	81.1	19.1	75.3	91.4
10	90	10.7	57.8[c]	91.8[a]	12.7	64.9	94.1[b]	29.7	80.0	99.0[b]
20	80	16.8	54.2[c]	90.2[a]	22.2[b]	63.0	96.6[b]	50.4[b]	84.7[b]	100.0[b]
30	70	25.6[b]	64.2[c]	93.9[a]	42.3[b]	88.8[b]	99.6[b]	87.7[b]	99.1[b]	100.0[b]
40	60	26.2[b]	69.5[c]	90.3[a]	56.5[b]	95.6[b]	99.8[b]	99.0[b]	100.0[b]	100.0[b]
60	40	47.3[a]	71.4[c]	91.9[a]	92.0[b]	99.0[b]	99.8[b]	100.0[b]	100.0[b]	99.9[b]
100	0	31.5	32.4	38.2	95.0	97.8	99.8	100.0	100.0	100.0
L.S.D. at 5% level		11.0	14.8	9.4	6.7	14.9	4.6	8.2	9.3	3.3
at 1% level		14.9	20.1	12.7	9.0	20.1	6.2	11.1	12.6	4.5
					Pupae					
0	100							10.0	17.2	45.0
10	90							17.0	23.0	71.5[b]
20	80							35.8[b]	48.4[b]	86.8[b]
30	70							61.2[b]	77.0[b]	96.4[b]
40	60							87.1[b]	95.2[b]	100.0[b]
60	40							100.0[b]	99.8[b]	100.0[b]
100	0							100.0	100.0	100.0
L.S.D. at 5% level								12.5	6.5	11.6
at 1% level								16.9	8.8	15.8

[a] Mixture more effective than either ethylene dibromide or methyl bromide.
[b] Mixture more effective than methyl bromide alone.
[c] Mixture more effective than ethylene dibromide alone.
(Comparisons made on the basis of L.S.D. values at the 1% probability level.)

about equally effective as fumigants, and all were more effective than either toxicant alone, on the basis of 1-day mortality data.

The superiority of the mixtures over the single toxicants, with respect to 1-day mortality, was very marked, especially at a dosage of 12 mg./l. For example, at this level neither toxicant alone gave as much as 50% kill of adults 1 day after exposure. By contrast, all of the mixtures killed over 90% of the adults in this period. The practical significance of this difference in speed of kill, between the mixtures and the single toxicants, cannot be fully assessed without further investigation. It could be important if fumigated adult beetles are capable of laying viable eggs in the period between their exposure to fumigant and their ultimate death from the exposure. If this were the case, reduction of the survival period could reduce the number of eggs laid. Preliminary observations on this point are discussed below.

Mixtures of ethylene dibromide and methyl bromide gave, with few exceptions, significantly higher kills of adults and larvae at 2 and 14 days after exposure, and of pupae at 14 days, than methyl bromide alone, but lower kills than ethylene dibromide (see per cent mortality values marked with superscript (b) in table 1). Even mixtures containing as little as 10% ethylene dibromide generally were much more effective than methyl bromide alone, at a given dosage level. For example, at 2 days after exposure, methyl bromide at 12 mg./l. killed only 52% of adults and 81% of larvae. At the same dosage rate the 10% ethylene dibromide/90% methyl bromide mixture killed 99% of adults and 94% of larvae. Mixtures containing 30 to 40% ethylene dibromide were, at the 10 and 12 mg./l. level, generally as effective as ethylene dibromide alone, on the basis of mortality at 2 or 14 days after exposure. In fact, in some instances the kill with the mixture was several per cent higher than that with ethylene dibromide, although these differences were not significant.

The more rapid kill of adults by mixtures as compared with that by the toxicants alone, especially at the higher dosage levels, was observed early in the investigation. This raised the question of whether surviving adults could lay viable eggs from which reinfestation could occur following fumigation. Accordingly, in subsequent experiments, observations were made on the effect of different fumigant treatments on the ability of surviving adults to lay viable eggs.

To determine whether viable eggs were present, the flour from these treatments in which one or more adult beetle had survived at least 2 days following fumigation was saved. After the 14-day mortality count, the adults, dead and alive, were separated from the flour on which they had been held. The flour was replaced in the original vial, with an additional portion of fresh flour, and incubated for 6 additional weeks. At the end of this time, the incidence of living larvae in each vial was determined. The number of adults surviving at any given mortality count, times the number of days since the last count, gave a value called, for convenience, "adult-days." These values were summed and averaged for each treatment. (Table 2.)

It is interesting to note that no larvae were found in vials where the adults had been fumigated with 1.0 mg./l.

Table 2.—Effect of fumigation on incidence of larval emergence in flour inhabited by adult confused flour beetles surviving treatment 48 hours or longer.

Composition of Fumigant (%)		Concentration of Fumigant (Mg./Liter)					
		8		10		12	
EDB	MB	Adult-Days[a]	Larvae Hatched[b]	Adult-Days	Larvae Hatched	Adult-Days	Larvae Hatched
0	100	1345	48	973	55	528	29
10	90	981	47	133	0	8	0
20	80	193	0	50	0	3	0
30	70	124	0	35	0	—[c]	—[c]
40	60	96	0	42	0	6	0
60	40	72	0	53	0	6	0
100	0	67	0	90	0	60	0
Control		1400	38	1386	13	1389	46

[a] Average number of days adults survived in each treatment. Five replicate tests were included at the 8 and 12 mg./l. level and two replicates at the 10 mg./l. level. A sample calculation of "adult-days" in a given treatment follows:

39 surviving adults at 24 hours—39×1 = 39 adult-days
21 surviving adults at 48 hours—21×1 = 21 adult-days
9 surviving adults at 14 days — 9×12 = 108 adult-days
Total 168

[b] Average number of emerging larva in each treatment.

[c] None of the adults survived to 48 hours following exposure.

or more of ethylene dibromide. Adults exposed to fumigation with a total of 0.8 mg./l. of ethylene dibromide, *i.e.*, 8.0 mg./l. dosage level of mixture containing 10% ethylene dibromide, were not affected with respect to the number of larvae subsequently produced. Treatment of the adults with methyl bromide alone likewise had no negative effect on the emergence of larva from eggs. There was no way of determining from this experiment the mechanism by which concentrations of 1.0 mg./l. or more of ethylene dibromide are effective in reducing larval emergence. Speculation could include selective killing of one sex, the creation of impotency in one or both sexes, malfunction of the egg-laying process, or laying of nonviable eggs. From the data in table 2, it would seem that mixtures containing methyl bromide and ethylene dibromide would have an added advantage over methyl bromide used alone in practical fumigation by preventing the rapid buildup of a new population from adults surviving fumigation. Commodities treated with these mixtures should thus remain free of insects for a longer period of time before reinfestation occurs.

DISCUSSION.—Methyl bromide has long been recognized as an effective fumigant for space and for stored products. Kenaga (1957) rated it as "excellent" with respect to toxicity to all life stages of grain insects, and "good" with regard to grain penetration. The same author also rated ethylene dibromide as "excellent" in toxicity, but "poor" with respect to penetration.

The high volatility of methyl bromide is an advantage from the standpoint of dispensing the toxicant and distributing it through the space to be fumigated. Ethylene dibromide, in contrast, has a low volatility and a relatively high freezing point (about 50° F.), making distribution difficult under certain circumstances. For these reasons, coupled with poor penetration properties, it has limited usefulness for application alone as a space or stored product fumigant, despite its high order of toxicity.

It would appear that the desirable features of both ethylene dibromide and methyl bromide may be utilized to practical advantage by the use of mixtures of the two fumigants. Because of its high volatility, methyl bromide can act as a propellant and dispersant for the mixture.

Composition of the mixture can be varied to suit the requirements of special fumigation problems, by varying the ratio of the components in the mixture. And, as the 2- and 14-day data in table 1 show, most mixtures in the range studied are substantially more toxic to insects than is methyl bromide, and at least equally as toxic as ethylene dibromide. This finding, coupled with the fact that the mixtures kill more rapidly than either fumigant alone, as shown by the 1-day mortality data in table 1, suggests that ethylene dibromide/methyl bromide mixtures have definite advantages as fumigants for storage space, stored products, and in related applications.

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A New Method for Sampling Arthropods Using a Suction Collecting Machine and Modified Berlese Funnel Separator[1]

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ABSTRACT

A high-speed motor fan was adapted to suck arthropods, along with some trash, from square-foot areas of an alfalfa field. The organisms were sucked into a collecting bag without damage, and these samples of leaves, duff, and living organisms were immediately refrigerated to retard predation and general exhaustive activity. At the end of the day the material from the sample bags was placed in modified Berlese funnels, which separated the organisms from the duff by entrapping the former in alcohol.

Three methods of using the suction collecting machine are discussed, along with their respective advantages and disadvantages. All data obtained will be presented in a separate paper at a later date.

A more nearly complete and quantitatively accurate sample of arthropods, from the ground surface to the top of the alfalfa stand, can be taken quickly in a single operation. These arthropods can also be separated by modified Berlese funnels into easily countable units in which the specimens are preserved. Modifications could make such machines useful in studying arthropods in other field and vegetable crops and possibly in tree crops.

During the last few years the authors have been working on the biological control of the spotted alfalfa aphid, *Therioaphis maculata* (Buckton). During these studies considerable time was spent in trying to measure the populations of all the arthropods associated with alfalfa, as well as the aphids and their natural enemies. The chief problem was the development of a standard sampling method that would gather all the organisms as completely as possible in order to obtain good estimates of the real or true populations.

The first model of the suction collecting machine was built in October, 1955, and was designed to collect large numbers of spotted alfalfa aphids without damaging them for use in the insectary production of the newly introduced parasites. The motor fan of the old model (fig. 1) was used to power this unit. The large aphid parasite collector used in the colonization program was a giant model of this early machine (van den Bosch *et al.* 1959). Neither of these machines provided a satisfactory quantitative measure of the arthropod populations.

In early 1958, prototypes of the suction collecting machine and the modified Berlese funnel separators were first tested and found to be efficient at quickly and completely gathering the arthropod fauna in hay fields. Other workers have obtained complete samples of arthropod populations. Hills (1933) reported the use of a suction pipette and a portable electric vacuum collector for collecting small insects from a square-foot counting cage. Hamlin *et al.* (1949) gathered complete square-foot samples of the alfalfa weevil, *Hypera postica* (Gyll.), from hay fields and laboriously sorted stems, trash on the ground, and soil samples. Eight such samples could be taken each week from each plot.

Johnson *et al.* (1955) adapted a portable electric blower with a 1-inch suction hose for use in collecting organisms from a square-foot cylinder which had been pressed into the grass. In their machine a nylon collecting bag, which fitted inside a barrel 10 inches long and 5.5 inches in diameter, was used for a sieve. However, the idea of using a cloth bag with which to sieve the organisms was developed independently by the authors.

SUCTION COLLECTING MACHINE.—The first machine (fig. 1) was made mostly from war-surplus materials. The improved collecting machine (fig. 2) cost approximately $250 and is composed of the following parts: (1) a portable fan, (2) a flexible air conduit, (3) collecting head and bag, (4) adapter hose, and (5) the mount.

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[2] The authors wish to acknowledge the technical assistance of Mr. Louis H. Dawson in the development of the suction collecting machine.

The Motor Fan.—The model 20B Homelite blower (Homelite: a Division of Textron, Inc., Port Chester, New York) used as an exhaust fan is lightweight and compact, with a single-cylinder, air-cooled, two-cycle gasoline engine. Variable air volumes from 500 to 1500 c.f.m. by engine throttle create a static pressure up to 5.1 inches of water. The overall dimensions are as follows: length, 18 inches; width, 17 inches; and height, 17½ inches. The engine and fan weigh 40 pounds. The motor fan for the original, old machine (fig. 1) was a larger, heavier, less efficient model.

Flexible Air Conduit.—The old machine had a custom-

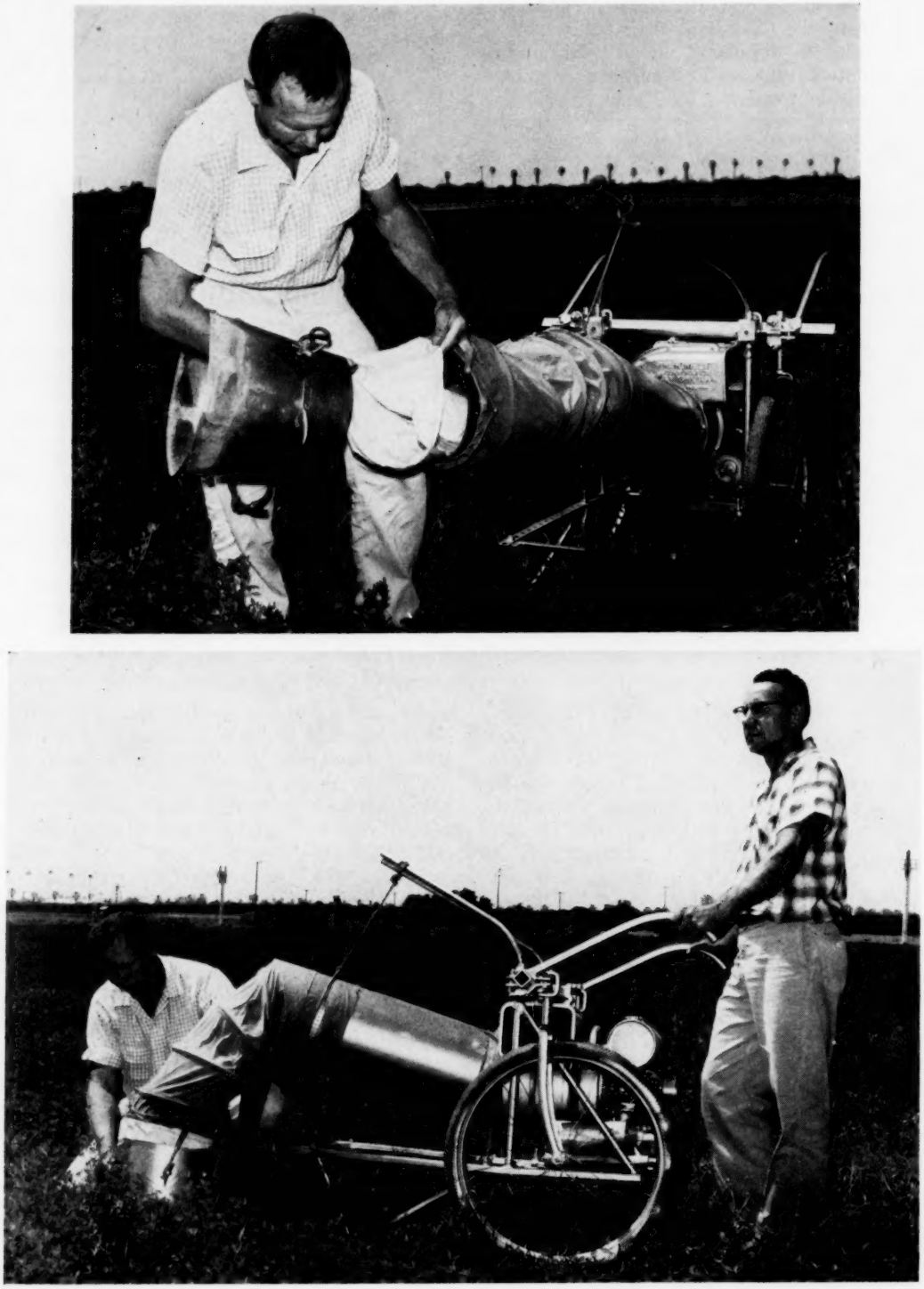

FIG. 1.—First model of suction collecting machine with bicycle-wheeled carrier.

made canvas conduit that was held open with wire ribs. The second model, or new machine, has a flexible non-collapsible conduit made of plastic-coated canvas. It is 14 inches in diameter and 15 feet long when stretched out but may be collapsed accordion-like into a 36×36×14-inch package that may be carried easily. When the air

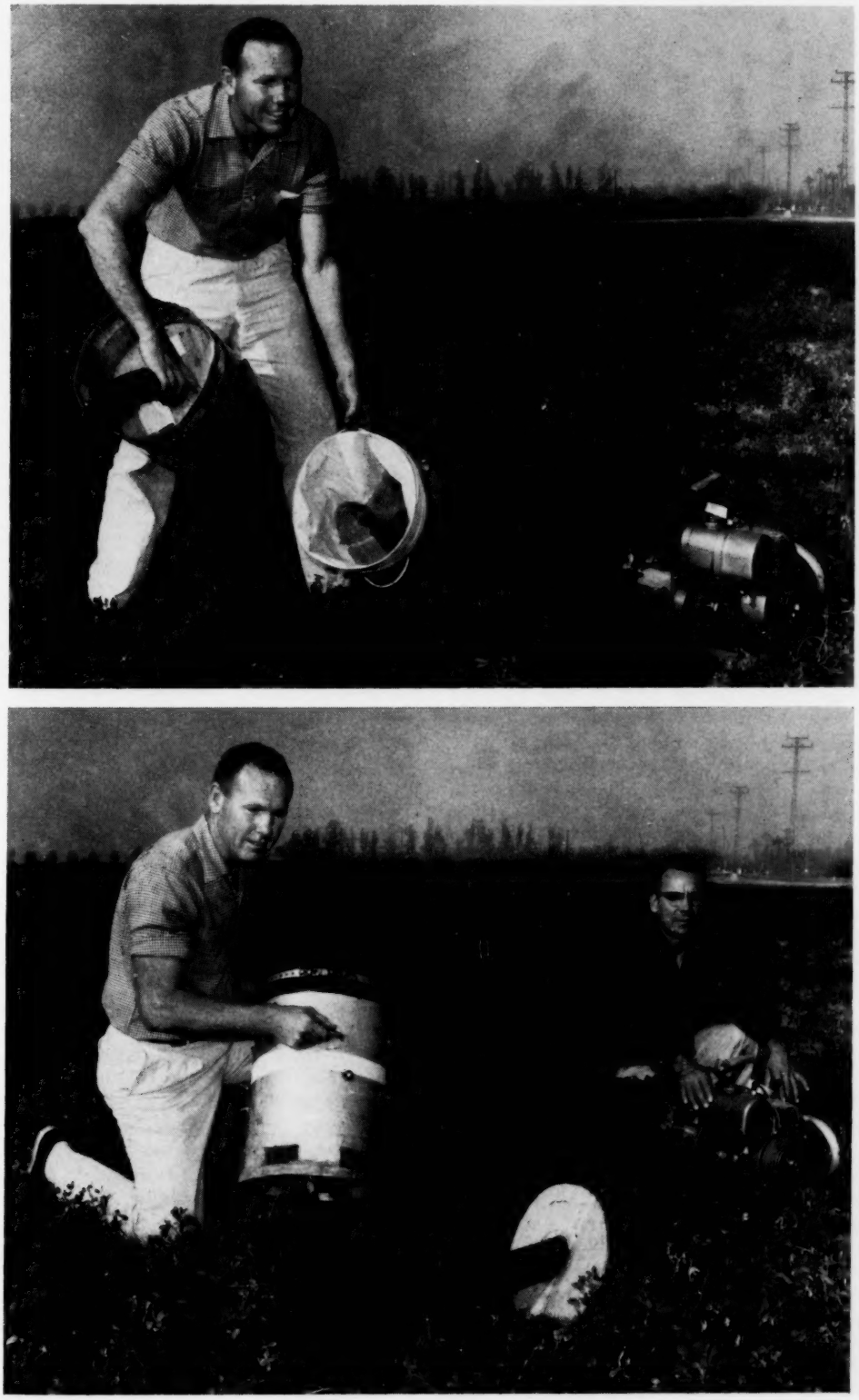

FIG. 2.—New model of suction collecting machine.

conduit is extended during the sampling, the organisms may be collected without disturbance from the motor or fan exhaust.

Collecting Head and Bag.—The collecting head confines the airflow from the area to be sampled and holds the collecting bag in place. The head is made of two lightweight aluminum cylinders, one of which is permanently connected to the air duct. The other, unattached cylinder, has an opening exactly 1 square foot in area, and fits inside the connected cylinder. The open collar of the collecting bag is held in place between these two cylinders. The bags are made of organdie and are fashioned to fit inside the connected cylinder and extend inwardly about 18 inches. The open collar is reinforced with muslin and is fitted over the outside of the connected cylinder. The bags are tapered to a smaller circumference at the closed end to allow the air to sieve through when the sack begins to fill with leaves and trash.

The open end of the unattached cylinder is ringed with 2- by 4-inch openings covered with fine-mesh screen, so that when the collecting head is held against the ground, a continuous flow of air is maintained around the plants. This is necessary to gather the organisms efficiently and to hold them and the trash firmly in the bottom of the sack, regardless of the position of the collecting head. This minimizes injury to the living organisms that are collected. Because there is considerable pull on the bag by the air and because of the physical manipulations in taking the samples, the unconnected cylinder is held in place by two heavy-duty rubber bands. These bands allow for quick and easy exchange of the collecting bags between sample replicates.

Adapter Hose.—The adapter hose is a 3-foot-long piece of flexible, noncollapsible, automobile air-duct hose with a plywood expansion-type connective fitting that is inserted into the open end of the collecting head when taking the complete type of sample. This hose measures 3 inches in diameter. The primary function of the adapter hose is to increase air velocity and hence suction power, in order to gather all the trash and loose dirt. The air chamber behind the hose inside the collecting head then acts as a deceleration chamber which keeps the increased suction from injuring the organisms as they are picked up with the duff.

The Mount.—Since the new machine weighs only 40 pounds, it has not been necessary to use a mount except when extensive plot experiments were undertaken. An assistant carries the motor fan by its handle from place to place, while the operator takes charge of the collecting head and air duct. The old machine was heavier, and the bicycle-wheeled cart (fig. 1) was pushed by one person while another raised and lowered the collecting head. The new machine can be placed on the cart in the same manner as the old machine.

MODIFIED BERLESE FUNNEL.—The successful completion of this sampling technique depends upon gathering the organisms in such a manner that they are kept alive. The samples were adequately refrigerated by placing prefrozen canned ice or re-usable ice packs in portable iceboxes. Overcrowding of the samples in these boxes may result in injury to the living organisms. When handled carefully, however, the organisms may be held for at least 8 hours before being transferred to the Berlese funnels. A very short exposure to carbon dioxide anesthetized the organisms sufficiently to transfer the material from the collecting bag to the Berlese funnel.

The Berlese funnel modified for this work (fig. 3) consisted of a metal cone 18 inches tall, with a diameter of 18 inches at the large end and of $2\frac{3}{4}$ inches at the small end, which was connected to a widemouthed pint fruit jar. The cover was a cone-shaped aluminum reflector 20 inches in diameter at the bottom and 8 inches high, with a hole 3 inches in diameter in the top, through which a 50- or 100-watt light bulb was inserted. A piece of black muslin was placed over the large end of this cover. When this cover was placed over the open top of the funnel and the bulb was inserted into the small opening of the cover, the heat from the bulb warmed the sample. The organisms were confined to the funnel and were not burned by contact with the bulbs. The weight of the metal cover sealed off the top of the funnel, and the jar closed off the bottom, forming an insect-proof container. The jar contained about 1 inch of alcohol which trapped and preserved the arthropods.

To prevent duff from falling into the alcohol along with the arthropods, trash trays were used. The trash trays mere cut from $\frac{1}{2}$-inch wire hardware cloth and had 3-inch strips of manila folder cardboard stapled to them. The upper tray was 16 inches in diameter and had four strips $1\frac{1}{3}$ inches apart. The lower tray was 15 inches in diameter and had three strips covering the spaces between the strips of the upper tray (fig. 3). A distance of 2 inches separated the two trays.

The material from the collecting sack was spread evenly over the cardboard strips before the cover was placed in position. Fifty-watt bulbs were lighted for the first 12 hours, then replaced by 100-watt bulbs. Excessive heat killed many organisms before they could be forced down away from the heat into the lower part of the funnel where they were eventually trapped in the alcohol. More heat was needed later to force the heat-resistant or negatively geotropic species from the protection of the trash. The samples were always run for 24 hours, though almost everything was trapped after 15 hours. Successive jars of clean alcohol were used to check the completion of the separation process. Samples containing a large amount of trash were divided between two funnels in order to keep the amount of material spread sufficiently for the Berlese separator to work efficiently.

Examination of the trash after the separation revealed that certain positively phototropic species were not getting down into the alcohol. Improvement of this part of the separation has resulted from some recent modifications in the Berlese funnel. These modifications have been tested sufficiently to justify description here.

No change has been made in the suction collecting machine, because the balance between the maximum gathering efficiency and the least injury to the organisms has been attained. The Berlese funnel has been blacked out except for a beam of light from a 75-watt spotlight directed upward from below the alcohol jar through the small end of the funnel. These lights are controlled by an automatic timer which turns them on and off every 15 minutes, because constant light from these bulbs overheated the alcohol in the jars and caused excessive evaporation of it. When the lights were on there was a positive

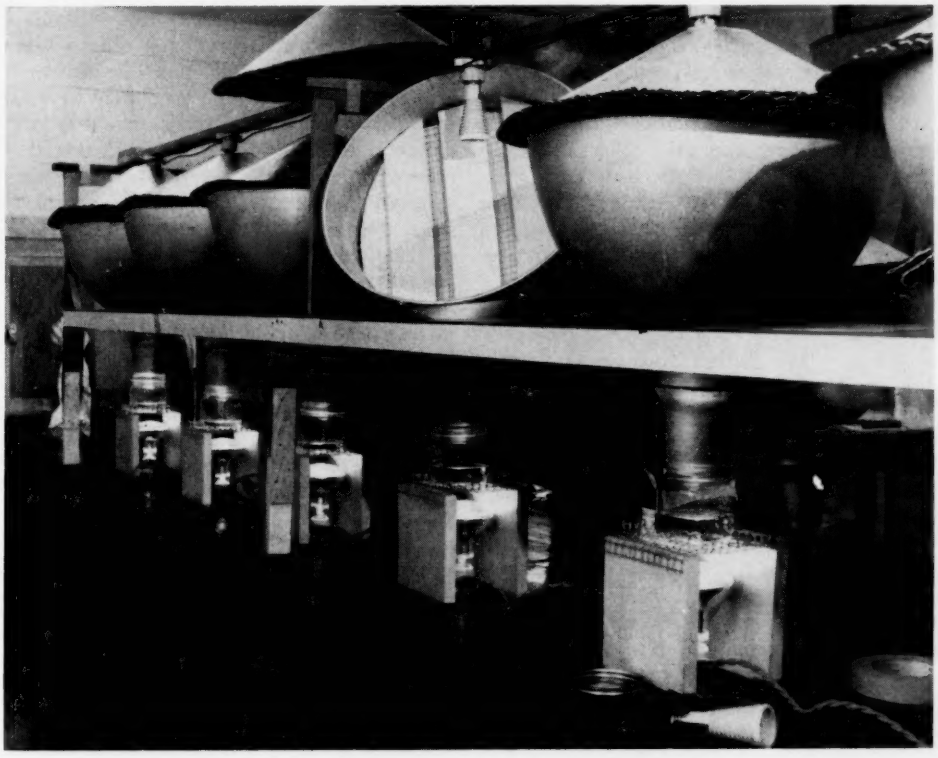

FIG. 3.—Berlese funnel separators.

phototropic gradient toward the alcohol trap.

The material from which the trash trays were made has been changed from cardboard to transparent plastic-impregnated wire screen. The light bulbs at the top have been replaced by 75-watt glocoil replaceable heating elements. These elements heat the samples slowly until a temperature of 120° F. is reached at the level of the trash and eliminate the need for light as a source of heat. The wattage is so low that there is no glow from the coils. "Powerstats" are used to control the amount of heat applied to the units. The voltage was increased from 60 to 110 volts after the first 8 hours. Thus, a gradient of light and heat, as well as any normal positive geotropic response, caused the organisms to leave the duff and become trapped in the alcohol.

It is important to keep the amount of trash to a minimum and spread out enough to allow the light from below to shine through to the top. The larger flying species are trapped completely only if lighted open areas extend through the duff.

EXAMINATION AND COUNT OF ARTHROPODS IN ALCOHOL.—One of the advantages of the Berlese method over other collecting methods is seen in the perfect condition of the preserved specimens, which are free of leaves and trash. Being preserved in alcohol, all or part of a sample can be counted immediately or set aside for future reference. The major portion of time can then be spent in counting in the laboratory, where the identification of the species is completed under most favorable conditions, with the aid of a stereoscopic microscope.

Five-bank laboratory counters are useful for tabulating the more numerous species. The greatest timesaver is a disk-type recording machine (Gray Audograph, Keynoter D-6) which uses moderately priced disks. The experienced counter, who knows the organisms, counts orally into the microphone. Later, the disk is played back and the count tabulated by less experienced technicians or clerical help. With this mechanical aid, the maximum part of the trained counter's time then can be devoted to his specialty. It is also possible to count large numbers and kinds of organisms with the same accuracy and speed as just a few species. By turning the recorder on only when voicing the count, 2 hours' counting can be recorded on a 20-minute disk; playback time is thus greatly reduced. There is probably a 50% saving in time by using the recording machine.

KINDS OF SAMPLES AVAILABLE.—By changing the manner in which the square-foot collecting head is placed in the hay, several types of samples can be taken. Three types are described: (1) the complete or faunistic, where the adapter hose is used; (2) the standard or straight air suction; and (3) a modified method.

The Faunistic Method.—This complete method was used to gather all possible organisms enclosed by the collecting head each time it was placed over the hay. The assistant placed the motor fan on the ground while the operator extended the flexible air duct away from the motor noise and air exhaust. The operator placed the collecting head over an area of undisturbed hay and pushed it quickly to the ground. The assistant then held the collecting head in place while the operator, using a pair of grass shears, cut away the plants surrounding the

collecting head, leaving a small clearing. Next, the collecting head was tilted backward just enough to allow the operator to cut the stems of hay contained within. These stems were shaken to help the airflow dislodge any organisms held tightly in the leaves and flower heads before they were withdrawn from under the collecting head. Upon completion of this act the collecting head again was pressed to the ground over the exact outline of the sampled area. Then, as the assistant raised the collecting head, the operator placed the plywood expansion fitting of the adapter hose into the open end. The suction of the machine helped to hold this in place, and with the help of the assistant, who still held the collecting head, the operator, using the adapter hose, gathered all the trash and organisms from the marked area before sampling the next square-foot unit.

The assistant and the operator were careful to hold the collecting head well above the hay and pointed upward when moving between the sampling operations, so that organisms would not be collected from outside the designated areas. Since the majority of the flying and jumping organisms were gathered by the first placement of the collecting head over the hay, there was very little loss of these kinds of specimens during the other phases of the sampling operation. Those individuals which had dropped to the ground were gathered quickly by the adapter hose, along with everything on the ground, in the crowns of the plants, at the base of the tillers, and even from cracks in the soil.

The limitations of this complete collecting method were associated with the large amount of trash and soil picked up. The amount of soil gathered was minimized by taking the sample when the ground was damp, but the trash was always present to some extent. Usually, only five individual square-foot units could be gathered in one sample before the collecting bag had to be changed. Changing this bag was no problem since the air flow held everything in the bottom, and no organism could fly or even crawl against the air movement. The operator removed the outer cylinder of the collecting head, while holding the collar of the bag firmly, and the bag was closed before the motor was stopped. With practice, a 5-square-foot sample can be obtained in 10 minutes.

Standard or Straight Air Suction.—First, the motor fan was set down in the field, and the flexible air duct was extended to face any wind that might be blowing. The collecting head, pointed upward, was carried with the air duct draped over the shoulder, except when a sample unit was to be taken. The operator then lowered the head over an area of undisturbed hay, put it quickly to the ground, and held it there for a few seconds. The air flowed through the screened openings around the base of the collecting head and upward through the stems, carrying with it some of the trash, most of the organisms, but very little of the soil particles or terrestrial organisms. The head was returned to the "up" position and one or two steps were taken in the arc formed by the air duct before a second unit was sampled. Generally, five such units were taken before the operator and his assistant moved the motor fan ahead to a new position. Great care was taken to keep motor speed and airflow constant.

Fifty or 100 square feet of area can be sampled before the trash affects the efficiency of this method. The small, lightweight species—those with irregular surfaces, the jumpers, fliers, and some that readily let go when disturbed—were easily collected. The heavier-bodied organisms and those that hung on tightly were not completely sampled. The fauna on the ground and among the bases of the tillers was only partially gathered. For the purpose of studying the aphids and their natural enemies, this sample gathered the aphids and the tiny parasites efficiently but missed some of the adults and larger larvae of the Coccinellidae. Consequently, a modified method was developed.

The Modified Method.—The operator handled the collecting head as described above, placing it over the hay and pressing it to the ground to mark the square-foot area. At this time he cleared away or pressed down the stems surrounding the outside of the collecting head. Then the operator, by tilting the collecting head backward with one hand enough to place his other hand underneath, sheared-off and shook the inside stems before removing them. When most of the stems had been cleared within the collecting head, the latter was returned to the ground a second time, covering precisely the same area. With the stems removed, almost everything was gathered from around the inside edge of the cylinder, as well as much of the material from the center of the circular area. The operator then quickly raised the collecting head to the "up" position and handed it to the assistant to hold while he examined the square-foot area. During this examination any coccinellids or larger organisms that had been missed were either counted and recorded on the identification tag for that sample or were placed, without damaging them, into the collecting head. During this quick examination there was some movement of the organisms into and out of the sample area, but the error was small and considered relatively insignificant. The last act before going on to the next square-foot unit was to place the collecting head over the area a third time in order to ensure collection of the maximum number of organisms.

ADVANTAGES AND DISADVANTAGES OF THE NEW METHOD.—A more nearly complete, quantitative sample of arthropods from the ground surface to the top of the alfalfa stand can be taken quickly in a single operation. These arthropods can also be separated by the Berlese funnels into easily countable units in which the specimens are preserved. Since more complete samples are gathered by the machine, it is much less affected by environmental factors such as time of day, temperature, wind, and moisture. For example, on cold, windy days when insect populations are low on the plant stems, the machine method works as well as on warm days, when the insects may be in the top part or canopy of the hay field. Comparable samples can be taken at any time of day or night and under many adverse weather conditions. Stubble or fields with very short hay growth can be sampled with the same accuracy as tall, full-grown hay. For many species of arthropods this appears to be the only way to gather enough units to make a quantitative study. The machine picks up all life stages of more species than any other method known to the authors. Therefore, the sample is not prejudiced by gathering only one stage of the life cycle. In host-parasite or predator population studies all of the associated species of arthropods are

gathered at the same time from within the identical sampling areas. Hence, correlations between any of the principle pests and their natural enemy species can be accurately measured.

The main disadvantage centers around the bulk of the machine and of the Berlese separators. Also, there will probably be mechanical problems and maintenance expenses as well as the problem of the initial cost of the machine and the Berlese separators.

CONCLUSIONS.—The two machines and modified Berlese separators are described primarily for the experimenting entomologist to use in quantitative population sampling of arthropods. It remains to be seen whether the suction collecting machine and Berlese separators will be used in everyday qualitative sampling by applied entomologists in place of or in addition to the insect sweep net or other sampling methods.

Modifications in the collecting head will make such machines useful in studying arthropods in other field and vegetable crops and possibly tree crops. All data obtained by these machines up to the present time will be presented in separate papers at later dates.

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Sorption of Single- and Multiple-Component Fumigants by Whole-Kernel Corn under Recirculation, and Correlated Mortality of Stored-Product Insects[1]

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ABSTRACT

Gas chromatography was used to determine the concentrations of acrylonitrile, carbon disulfide, carbon tetrachloride, chloropicrin, ethylene dibromide, ethylene dichloride, hydrocyanic acid, methyl bromide, and of fumigant mixtures (on a volume basis) of acrylonitrile-carbon tetrachloride (25-75), carbon disulfide-carbon tetrachloride (20-80), ethylene dichloride-carbon tetrachloride (75-25), and carbon tetrachloride-ethylene dichloride-ethylene dibromide (60-35-5), under conditions of no load and of a load of corn (whole-kernel grade No. 2 yellow) under recirculation of fumigant. Mortality of adults of *Tribolium confusum* Duv. and *Sitophilus oryza* (L.) are included.

Fumigation is one of the most effective methods of controlling stored-product insects. Such factors as sorption (both absorption and adsorption) by the commodity, temperature, exposure, and fumigant have a pronounced effect on fumigation results. It is well known that sorption varies with the fumigant used, the commodity being fumigated, and the temperature and moisture content of the commodity. The rate at which sorption takes place and the amount sorbed determine the amount of fumigant remaining in the gas phase and available to the insect that is being fumigated.

Vapor-phase chromatography is generally applicable to the qualitative and quantitative determinations of all components of gaseous and vaporizable liquid mixtures. This paper gives the results of investigations to determine (1) the sorption by corn (whole-kernel grade No. 2 yellow) of various single- and multiple-component fumigants as determined by gas chromatography, and (2) corresponding mortality of adults of *Tribolium confusum* Duv. and *Sitophilus oryza* (L.).

MATERIALS AND METHODS.—Nine recirculation fumigation chambers were used in this study. These chambers were set up in a room in which the temperature could be controlled within ±1° F. The fumigation chambers were gastight. The entire chamber had a volume of 53 liters, and, exclusive of the return-duct system, held 70 pounds of corn (whole-kernel grade No. 2 yellow) of 12% to 13% moisture content. Each chamber consisted of a galvanized metal tube 7 inches in diameter and 6 feet in length; the return duct consisted of a galvanized pipe, 1½ inches inside diameter. With no load, air was moved at the rate of nine changes per minute by a built-in multivaned fan. With a load of corn the rate of air movement dropped to one change per minute, not allowing for the space occupied by the corn.

Adults of *T. confusum* and *S. oryza* 2 to 3 weeks old were used in these experiments, and all experiments were replicated three or more times. Approximately 120 test insects of each species were confined in separate probes which were inserted into the center of the column of corn. After fumigation the test insects were held at a temperature of 80° F. and 60% relative humidity, in the presence of food. Mortality counts were made 7 days after fumigation; natural mortality was negligible in the controls.

A gas chromatographic apparatus (Model 154-C Perkin Elmer Fractometer) equipped with a gas-sampling valve was used for gas analysis. The gas sample (5 ml.) is inserted into a continuous stream of carrier gas (helium) which moves the individual components through a column

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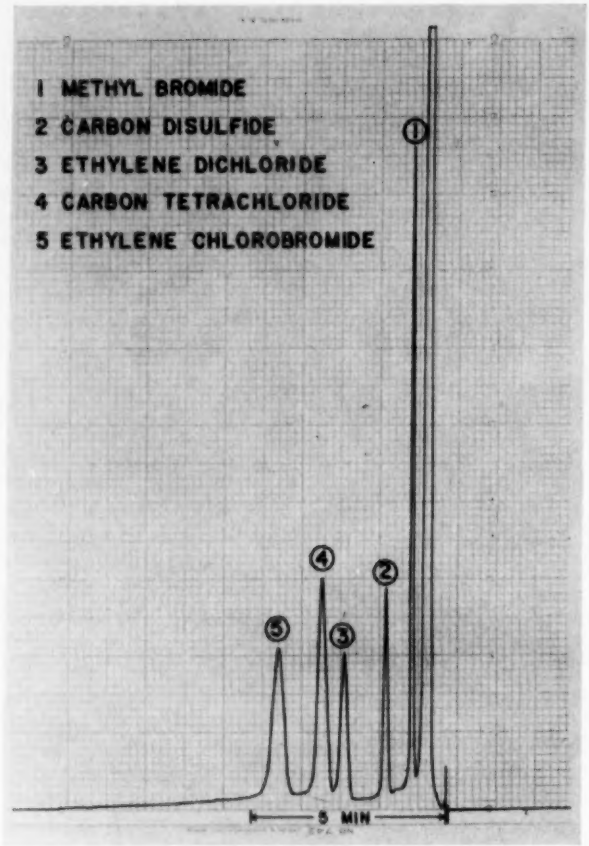

FIG. 1.—Gas chromatogram showing separation of a five-component gas mixture using a 5-ml. gas sample.

at different speeds depending on their respective retention by the column material. Each component therefore appears at the column exit at a definite time rate characteristic of that component's affinity for the column material used. If a suitable detector and recording system is placed at the exit of the column, a series of peaks will be recorded where the peak location is characteristic of the component and the area under the peak or peak height is a measure of the concentration.

A gas chromatogram of a five-component fumigation mixture consisting of methyl bromide, carbon disulfide, ethylene dichloride, carbon tetrachloride, and ethylene chlorobromide is shown in figure 1. The retention time indicates the component, while the peak area or height provides the quantitative measurement. By the use of various column packings, column lengths, column temperatures, and carrier gas pressure, the retention time is varied, and thus effects separation (Phillips 1956, Keulemans 1957).

Fumigants used in this study included acrylonitrile, carbon disulfide, carbon tetrachloride, chloropicrin, ethylene dibromide, ethylene dichloride, hydrocyanic acid, methyl bromide, and mixtures on a volume basis of (1) 25% acrylonitrile and 75% carbon tetrachloride; (2) 20% carbon disulfide and 80% carbon tetrachloride; (3) 75% ethylene dichloride and 25% carbon tetrachloride; and (4) 60% carbon tetrachloride, 35% ethylene dichloride, and 5% ethylene dibromide. The fumigants were applied as liquids, except methyl bromide, which was applied as a gas. The liquids were measured onto sorbent wicks which were suspended in the return duct directly in the air stream. Ethylene dibromide at higher dosages appeared to require more than 15 minutes for total evaporation. When high dosages were required for effective kill of insects, considerable positive pressure developed in the fumigation chamber during the evaporation period and was released. Later on, during the fumigation cycle, a negative pressure developed in the chamber as a result of the sorption factor. This, also, was released to maintain the fumigations at atmospheric pressure.

Exposure was for a period of 24 hours. By the use of a time clock which turned the fan off and on, the air was automatically recirculated in the chamber during the 1st, 6th, 12th, 18th, and 24th hours. When gas samples were taken during an interval when the chamber was not under recirculation, the fans were turned on manually for a few minutes prior to sampling.

Gas samples were taken at the 15-minute, 30-minute, 1-, 2-, 4-, 6-, and 24-hour interval in the presence of a load; in the empty chamber, gas samples were taken at the 30-minute, 6- and 24-hour interval.

Whole-kernel grade No. 2 yellow corn was the commodity fumigated. There was a certain amount of dockage in the corn, such as fragments of cobs, weed seeds, and dust, as well as a light-to-moderate infestation of rice and granary weevils. The moisture content of the corn was maintained between 12% and 13%. Seventy pounds of corn (1¼ bushels) were required to fill the fumigation chamber to capacity.

RESULTS.—The relative effectiveness of various fumigants against adults of *T. confusum* and *S. oryza* under conditions of load and of no load is shown in table 1. Dosages marked with a super-script capital letter are graphically illustrated in figures 2 A-H and 3 I-L to indicate the concentration obtained for the 24-hour exposure period. The amount of fumigant required to give comparable kills was greater in the presence of corn than with no load. Ethylene dichloride in the presence of no load was much more effective in killing adults of both *S. oryza* and *T. confusum* than was carbon tetrachloride alone, but in the presence of corn there was little difference between the two. These tests indicate that under conditions of no load, acrylonitrile, chloropicrin, ethylene dibromide, hydrocyanic acid, and methyl bromide are highly effective, but in the presence of corn, methyl bromide appeared to be one of the most effective of the fumigants used.

In comparing the fumigant mixtures on an approximately equivalent kill basis employing recirculation (table 1), the following observations can be made: (a) On the basis of gallons per thousand bushels of corn, the mixture by volume of 75% ethylene dichloride and 25% carbon tetrachloride is no more effective than carbon tetrachloride or ethylene dichloride alone; (b) the mixture by volume of 60% carbon tetrachloride, 35% ethylene dichloride, and 5% ethylene dibromide proved to be more effective than the mixture of 75% ethylene dichloride and 25% carbon tetrachloride on a basis of gallons per thousand bushels of corn.

The relative concentrations of various fumigants and

Table 1.—Effectiveness of various fumigant mixtures against adults of *Tribolium confusum* Duv. and *Sitophilus oryza* (L.) under recirculation of fumigant with no load and with a load of 70 pounds of whole-kernel corn.[a]

Fumigant	Formula	No Load: Fumigant Applied, Mg./l.	No Load: Kill (%) *T. confusum*	No Load: Kill (%) *S. oryza*	Load Whole-Kernel Corn: Fumigant Applied, Mg./l.	Load Whole-Kernel Corn: Gallons/1,000 Bu.	Load Whole-Kernel Corn: Kill (%) *T. confusum*	Load Whole-Kernel Corn: Kill (%) *S. oryza*
Acrylonitrile	CH_2:CHCN	0.37	0	14.5	9.0	0.13	0.3	52.2
		0.75[A]	7.0	100	13.5[A]	0.19	7.2	100
		3.0	100	100	18.0	0.25	71.5	100
Carbon disulfide	CS_2	11.8	5.0	50.0	47.4	0.42	0.8	10.8
		23.7[B]	74.8	100	94.8[B]	0.85	37.3	99.2
		47.4	100	100	142.2	1.27	97.6	100
Carbon tetrachloride	CCl_4	15.0	0	0.4	179.5	1.27	94.2	28.5
		60.0	84.9	34.2	359.0	2.5	97.4	76.2
		179.5[C]	100	94.4	539.0[C]	3.8	100	86.7
Chloropicrin	CCl_3NO_2	1.6[D]	5.3	98.9	15.5[D]	0.11	22.3	97.7
		3.1	90.8	100	31.0	0.21	100	100
		6.2	100	100	62.0	0.42	100	100
Ethylene dibromide	CH_2BrCH_2Br	2.0[E]	100	100	11.8	0.06	8.9	0
		4.1	100	100	23.6	0.13	79.3	54.8
		8.1	100	100	35.3[E]	0.19	95.5	97.6
Ethylene dichloride	CH_2ClCH_2Cl	11.8	80.3	4.6	141.5	1.27	88.9	0.5
		23.6[F]	100	22.0	283.0[F]	2.5	100	13.5
		47.2	100	90.3	424.0	3.8	100	35.5
Hydrocyanic acid	HCN	1.3	91.6	25.5	6.5	0.11	85.5	0.2
		6.5[G]	100	74.4	38.7	0.63	100	51.3
		12.9	100	100	64.5[G]	1.06	100	67.9
Methyl bromide	CH_3Br	1.1	0.3	14.1	3.6	0.023	0	3.5
		3.3[H]	100	100	7.1	0.046	2.6	100
		4.3	100	100	10.7[H]	0.07	95.7	100
Acrylonitrile	CH_2:CHCN	0.37			9.0			
Carbon tetrachloride	CCl_4	2.24	1.2	17.0	53.9	0.51	10.5	86.5
Acrylonitrile	CH_2:CHCN	0.75			11.2			
Carbon tetrachloride	CCl_4	4.5	4.5	99.5	67.3	0.63	32.3	99.2
Acrylonitrile	CH_2:CHCN	1.5[I]			13.5[I]			
Carbon tetrachloride	CCl_4	9.0	100	100	80.8	0.76	99.5	100
Carbon disulfide	CS_2	4.7			19.0			
Carbon tetrachloride	CCl_4	24.0	61.6	13.1	96.0	0.84	10.9	1.0
Carbon disulfide	CS_2	9.5[J]			38.0			
Carbon tetrachloride	CCl_4	48.0	100	96.0	191.5	1.69	81.5	58.8
Carbon disulfide	CS_2	14.2			57.0[J]			
Carbon tetrachloride	CCl_4	72.0	100	99.7	287.3	2.5	100	98.1
Ethylene dichloride	CH_2ClCH_2Cl	17.7			106.0			
Carbon tetrachloride	CCl_4	7.5	99.2	0	45.0	1.27	96.5	1.3
Ethylene dichloride	CH_2ClCH_2Cl	35.4			212.0			
Carbon tetrachloride	CCl_4	15.0	100	8.2	90.0	2.5	100	4.2
Ethylene dichloride	CH_2ClCH_2Cl	53.1[K]			318.0[K]			
Carbon tetrachloride	CCl_4	22.5	100	87.4	135.0	3.8	100	26.7
Carbon tetrachloride	CCl_4	4.5			108.0			
Ethylene dichloride	CH_2ClCH_2Cl	2.1	54.2	14.7	49.5	1.27	100	12.0
Ethylene dibromide	CH_2BrCH_2Br	0.5			12.2			
Carbon tetrachloride	CCl_4	9.0[L]			161.6[L]			
Ethylene dichloride	CH_2ClCH_2Cl	4.1	100	93.9	74.3	1.9	100	91.2
Ethylene dibromide	CH_2BrCH_2Br	1.0			18.3			
Carbon tetrachloride	CCl_4	18.0			215.5			
Ethylene dichloride	CH_2ClCH_2Cl	8.2	100	100	99.1	2.5	100	100
Ethylene dibromide	CH_2BrCH_2Br	2.0			24.4			

Moisture content of corn, 12% to 13%. Exposure: 24 hours at 70°F.

[a] Superscript capital letters refer to corresponding lettered graphic illustrations in figures 2 A–H and 3 I–L.

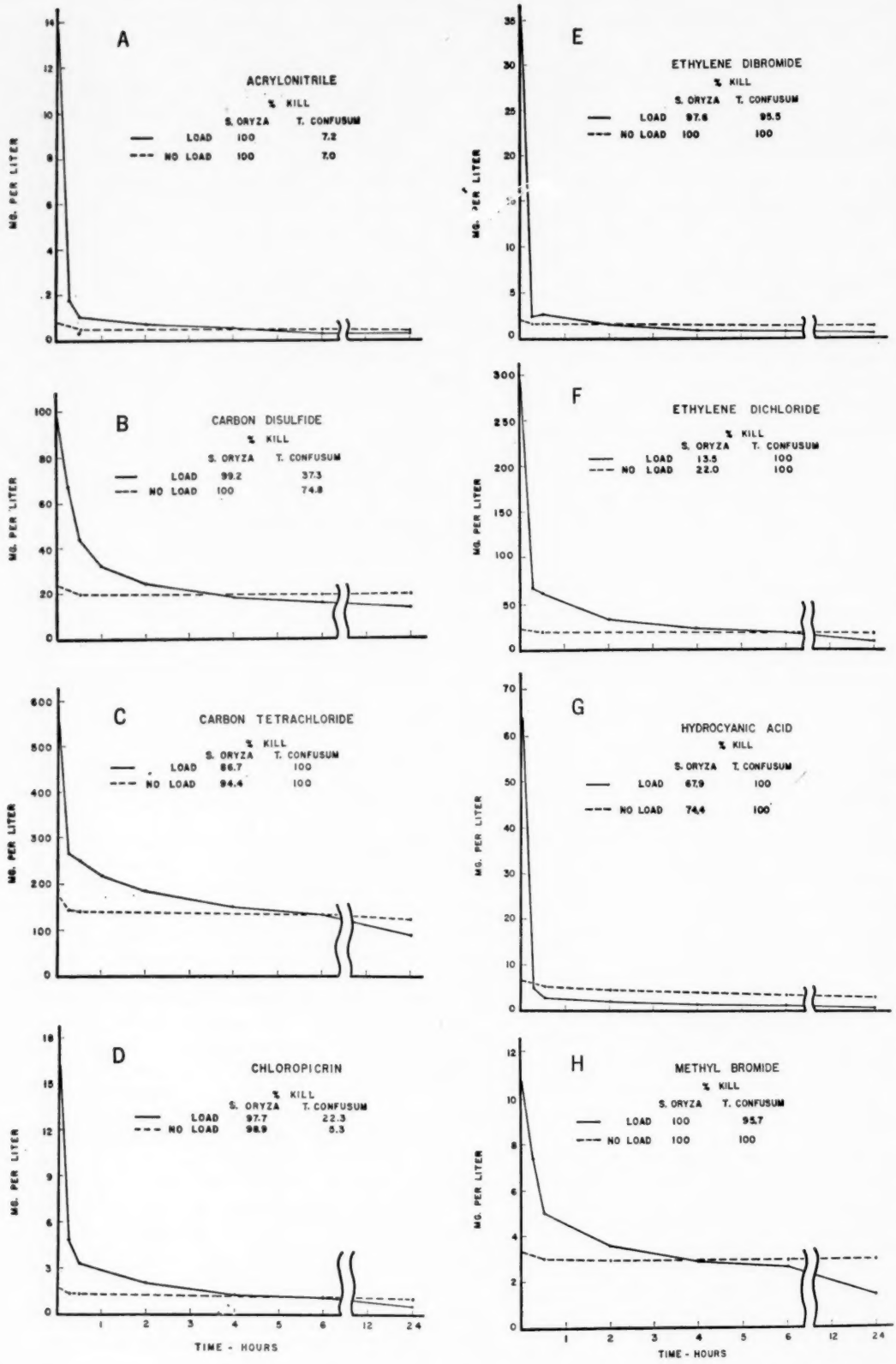

FIG. 2.—Concentration of fumigants and mortality of adults of *Tribolium confusum* and *Sitophilus oryza* under recirculation of fumigant with no load and with a load of 70 pounds whole-kernel corn (12%–13% moisture content). Exposure: 24 hours at 70° F.

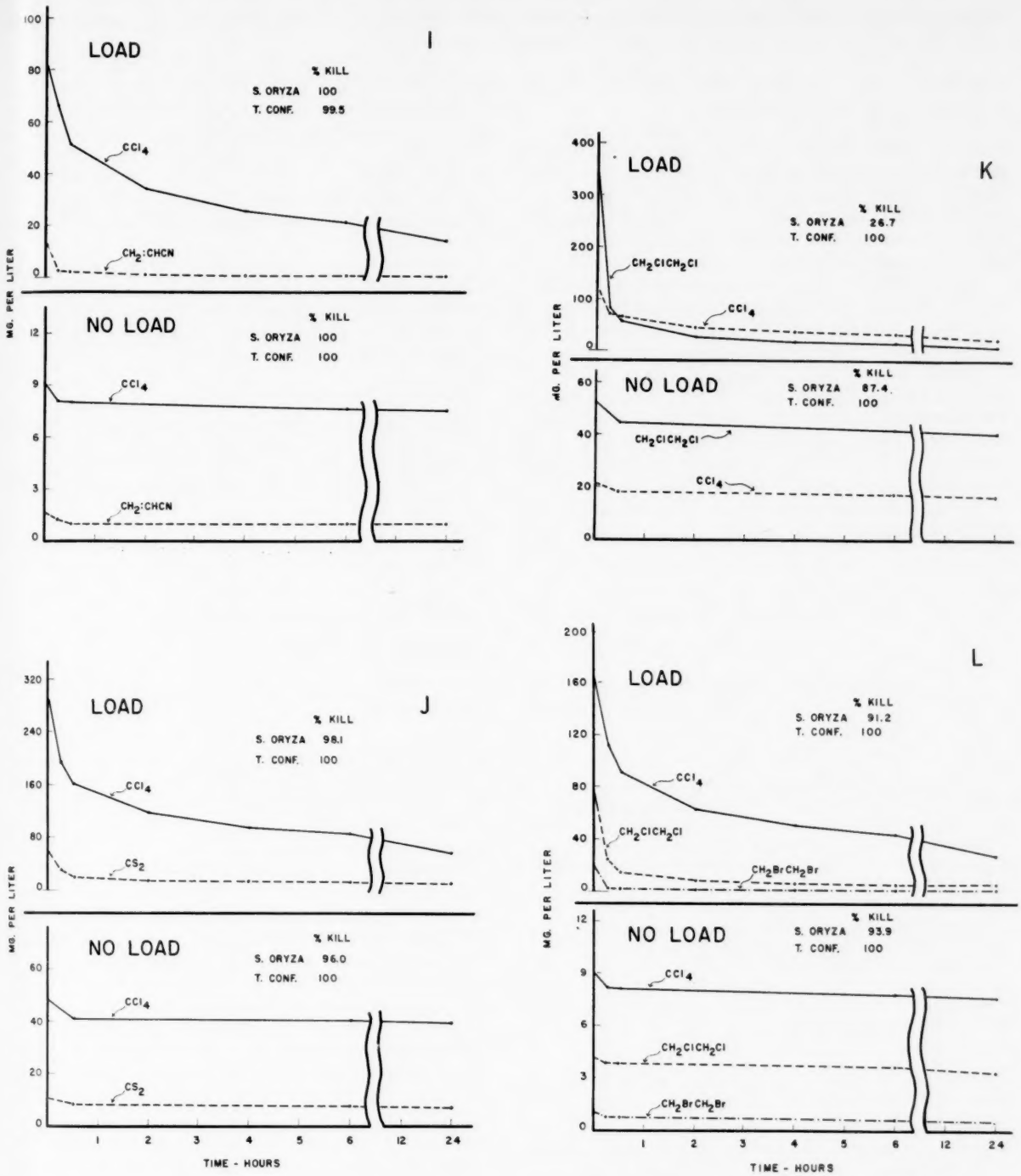

FIG. 3.—Concentration of components of fumigant mixtures, and mortality of adults of *Tribolium confusum* and *Sitophilus oryza* under recirculation of fumigant with no load and with a load of 70 pounds of whole-kernel corn (12% to 13% moisture content). Exposure: 24 hours at 70° F.

mortalities of adults of *T. confusum* and *S. oryza* are shown in figures 2 A-H and 3 I-L. These graphs show the dosage applied and the gas concentration obtained at sampling intervals for the various fumigants under conditions of load and no load and are compared on the basis of similar insect mortality.

The concentration of fumigants and each component of the fumigant mixtures obtained under conditions of no load varied little between the first and final sampling periods. A slight initial drop in concentration occurred prior to the first sampling interval (figs. 2 and 3). In the presence of corn the greatest reduction in concentration

occurred during the first 15 minutes following application of the fumigant, indicating a very rapid rate of sorption by the corn. With some of the fumigants, this drop in concentration may have been more rapid than the 15-minute sampling interval indicates.

The initial drop in concentration in the presence of corn was greater for acrylonitrile, ethylene dibromide, and hydrocyanic acid (see fig. 2 A, E, G) than for carbon disulfide, carbon tetrachloride, and methyl bromide (see fig. 2 B, C, H). This indicates that carbon disulfide, carbon tetrachloride, and methyl bromide are sorbed less rapidly by corn than are the other fumigants tested.

Figure 3 I-L shows the concentrations obtained with various fumigant mixtures. In the mixture (by volume) of 25% acrylonitrile and 75% carbon tetrachloride, the concentration of acrylonitrile dropped from 13.5 mg./l. to 1.8 mg./l. or 87%, in 15 minutes, and by the end of 24 hours was less than 0.5 mg./l. in the presence of corn; whereas the decrease in concentration of carbon tetrachloride was slower, and dropped from 81 mg./l. to 67 mg./l., or 17%, in the first 15 minutes and at the end of 24 hours was approximately 14 mg./l. (fig. 3 I).

The concentration curves (fig. 3 J) for the mixture of 80% carbon tetrachloride and 20% carbon disulfide indicate that the rate of sorption by corn was similar for both components.

Figure 3 K illustrates the differential rate of sorption by corn of a mixture of 75% ethylene dichloride and 25% carbon tetrachloride. The ethylene dichloride component of this mixture dropped from 318 mg./l. to 88 mg./l., or 72%, in the first 15 minutes, and at the end of 24 hours was approximately 11 mg./l., while the carbon tetrachloride fraction dropped from 135 mg./l. to 80 mg./l., or 41%, in the first 15 minutes and at the end of 24 hours was 22 mg./l.

In the mixture by volume of 60% carbon tetrachloride, 35% ethylene dichloride, and 5% ethylene dibromide, the drop in concentration in the presence of corn was greatest for ethylene dibromide and least for carbon tetrachloride (fig. 3 L).

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The Residual Insecticidal Effect of Bayer Compound S-1752 (Bayer 29 493) in Laboratory Tests[1]

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ABSTRACT

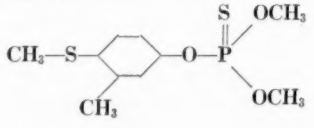
Chemical, physical and biological properties of a new residual insecticide namely Bayer S-1752 (29 493) (*O,O*-dimethyl *O*-(4-methylthio-*m*-tolyl)phosphorothioate) are described. Under laboratory conditions residues of S-1752 were tested on glass plates and sun-dried mud against flies (*Musca domestica* L.), triatomids (*Rhodnius prolixus* Stahl) and mosquitoes (*Culex p. quinquefasciatus* Say).

Varying exposure and observation times according to the testing animals were used. The results showed that the duration of persistence of S-1752 depends upon the absorptivity of the treated surface.

No difference in efficacy of S-1752 was found against susceptible or DDT-resistant house flies.

As part of the research work of the Insecticide Department of the Ministerio de Sanidad y Asistencia Social, Maraquay, Aragua, Venezuela, laboratory tests were made on the residual effect of a new insecticide included in the phosphorus compounds. The results of these tests are summarized in this paper. The corresponding field tests will be the theme of a later report.

METHODS AND MATERIALS.—The compound, identified as Bayer S-1752 (Bayer 29 493), *O,O*-dimethyl *O*-(4-methylthio-*m*-tolyl)phosphorothioate has the following structural formula,



It is a brown liquid, soluble in most organic solvents and practically insoluble in water; it is thermally stable up to 210° C. and resistant to alkali up to pH 8.[2] Its vapor pressure at 20° C. is $2.15 \cdot 10^{-6}$ mm. Hg. It is extraordinarily resistant to light.

The experiments summarized here were carried out under laboratory conditions. The insects were exposed for a certain time to surfaces previously treated with S-1752, and their subsequent behavior was observed. We used the following insects bred in our laboratories: a) House flies (*Musca domestica* L.) belonging to two strains. One strain, considered susceptible, bred without any intentional pressure from any insecticide. Another strain, R-DDT, developed from the former by progressive pressure of DDT on the adult stages of the insect through 64 generations. In the experiments we used 3- to 8-day-old flies without distinction of sex, in groups of 20 to 30 per replicate. b) Triatomids (*Rhodnius prolixus* Stahl) belonging to a colony established in 1948, with insects captured in a neighboring area, which at that time had never been treated with insecticides. We used fifth instar nymphs, 2 to 4 days after their last ingestion of blood, in numbers that varied from 10 to 20 per replicate. c) Mosquitoes (*Culex p. quinquefasciatus* Say) from a colony kept in our laboratories, free from intentional contacts with insecticides, and started in 1948 with females cap-

[1] Accepted for publication May 29, 1959. Partial cost of publication of this paper was met by Metachem Inc., 425 Park Ave., New York 22, N. Y.

[2] Concerning the water-soluble part (10 p.p.m.), higher concentrated suspensions up to pH 12.5 and more.

Table 1.—Per cent mortality in *Musca domestica* laboratory strains (S) susceptible and (R) resistant to DDT after a 24-hour observation period after exposures of 5, 15, or 30 minutes on various surfaces sprayed with suspensions of a wettable powder of Bayer S-1752 at the rates of 1 and 3 gm. active ingredient per sq. meter.

Weeks After Treatment	1 Gm./M.²												3 Gm./M.²											
	Glass				Sun-Dried Mud				Sun-Dried Mud			Lime-Coated			Sun-Dried Mud						Sun-Dried Mud Lime-Coated		
	S		R		S				S			R			S			R			S		
	5	15	5	15	5	15	30		5	15	30	5	15	30	5	15	30	5	15	30	5	15	30
1	100	100			79.2	77.5	85.5		85.6	72.7	100				97.4						93.6		
2	100	100			70.2	78.4	81.2		79.5	84.8	96.9				80.6	80.8		75.9	86.7		66.6	80.7	
3	100				63.7	49.3	55.9		70.4	55.7	80.				100	52.7	63.0	47.1	65.5	53.1	98.4	62.0	
4	100				30.9	28.4	34.		27.4	27.2	38.2				16.7	85.	28.0	21.2	13.0	23.6			
5	100		100		71.2	59.1	76.5		62.	68.4	77.6	23.0	25.	27.2							43.2	49.8	27.1
6	100				23.6	25.3	23.6		28.	27.2	19.4				23.4	35.5	100	11.6	14.6	33.3	11.4	29.	60.
7	100		100		41.3	22.4	78.8		55.2	46.4	44.4	25.7	30.1	41.8	19.6	21.6	35.3	0	12.5	28.5			
8	100						0		0	12.9	24.3				15.6	30.1	23.0	26.7	37.2	19.3			
9	85.														1.6								
10	100		100												1.6	14.2	21.8						
11	85.9				41.5	16.7	40.		26.9	31.5	35.5				3.5	4	5						
12	86.	77.3	100		27.7	18.	31.6		42.8	45.4	43.6							13.7	12.9	12.			
					12.7	15.1	9.2		48.	54.2	38.6	17.5	6.	17.3									
13	70.	79.3	95.5		13.1	12.7	14.2		22.4	86.6	25.4							0	0	0			
14	45.8	78.9	44.8	56.8	19.6	10.6	16.0		11.8	30.9	32.7				9.5	18.1	10.						
15	57.8	41.2			0	0	0		2.4	5.1	0	0	0	0									
16					0	0	0		0	5.6	1.8												
17					0	3.6	4.0																
18					0	3.8	6.8																
19					0	0	0																

Table 2.—Exposure time required[a] to obtain 50, 90, and 100% "knockdown" in *Musca domestica*, of a normal laboratory strain, exposed continuously to various surfaces, sprayed with a wettable powder of 20% Bayer S-1752, at rates of 1 or 3 gm. of active ingredient per sq. meter.

	1 GM./M.²									3 GM./M.²					
	Glass			Sun-Dried Mud			Sun-Dried Mud Lime-Coated			Sun-Dried Mud			Sun-Dried Mud Lime-Coated		
DAYS AFTER TREATMENT	50	90	100	50	90	100	50	90	100	50	90	100	50	90	100
1				1–23	1–15	1–45	0–28	0–41	0–50						
5										0–55	1–20	1–30			
6							1–50	4–10	5–20				0–47	0–62	0–85
9				3–45	5–50	6–40	2–29	4–40	5–40						
16				4–20	6–25	7–0	3–55	5–15	7–0						
21										1–20	1–45	2–10	1–15	1–36	2–05
26				6–10			5–40	7–05							
29				5–10			4–10	6–10							
33				5–15			7–20								
34	0–16	0–20	0–25												
40				4	7–0		7								
41	0–17	0–25	0–30												
47				7–15			7–10								
48	0–14	0–23	0–30												
61	0–40[b]	0–58	0–65												
65				7–10			7–0								
71							6–30[b]								
76	0–58	1–32	1–40												
76	0–19[c]	0–28	0–35												

[a] Time expressed in hours and minutes.
[b] Much used glass plate.
[c] Glass plate not used before.

tured in Puerto Caballo, before the beginning of the campaign with chlorinated insecticides. In the present experiments we used females fed on pigeons 5 to 15 hours before their exposure to S-1752 in groups of 15 to 20 insects per replicate.

We treated the following types of surfaces: a) glass plates measuring 20×20 cm., previously used and washed in recent months and therefore considered of neutral reaction; b) wooden frames with inside measurements 20×20 cm., and filled with two materials used in rural constructions: 1) adobe, sun-dried mud; and 2) "encalado," the same material which, after drying, was covered with a thick layer of lime and water. The glass plates and the blocks were treated with suspensions of the Bayer compound S-1752, formulated as a wettable powder containing 20% active ingredient.[3] Application was made in such a way as to obtain deposits of 1 or 3 gm. of active ingredient per square meter.

The insects were exposed to the treated surfaces, groups confined under petri dishes (90 mm. diameter) for periods previously agreed upon, and transferred for observation to paper cages. The usual techniques, developed in our laboratory and described in previous papers, were applied.

The length of exposure and observation varied according to the insects. In the case of the flies two types of tests were made: a) contacts of 5, 15, and 30 minutes, respectively, with check on mortality after 24 hours; and b) continuous contact with periodical checks on knockdown. In the triatomid tests the exposures were 10, 30, and 60 minutes, respectively, with daily checks on knockdown over a period of 10 days. The mosquitoes were exposed for 5, 15, or 60 minutes, and the mortality rate was established after 24 hours.

RESULTS.—*Test with flies.*—Table 1 shows the per cent mortality observed 24 hours after exposure to the insecticide for a pre-established period for susceptible as well as for resistant flies. In all tests high mortality was registered during the first weeks; this record of mortality was maintained for 14 weeks in the glass-plate tests, but decreased spasmodically in the tests on materials such as adobe and encalado at dosages of 1 gm. or 3 gm. active ingredient per square meter.

Similar effects, observed in table 2, resulted on glass-plate deposits, where a rapid knockdown following short contacts was continued up to 76 days, while on adobe and encalado the time of exposure necessary to obtain 50, 90, or 100% mortality increased rapidly. It took more than 7 hours to obtain 50% knockdown 65 days after treatment of the blocks.

The persistence of the insecticidal effect on flies on glass surface is noteworthy, and shows no considerable decrease after 12 weeks, or after 76 days (see tables 1 and 2, respectively). The loss of activity expressed by the increase of the exposure time necessary to obtain the knockdown effect after 61 and 76 days, as shown in table 2, was due to mechanical removal of the deposit in the course of previous tests with the same plates.

Tests with triatomids.—Table 3 indicates the per-

[3] Formulation Bayer 4894, provided by the Biological Institute of Farbenfabriken Bayer, Leverkusen, Germany.

Table 3.—Percentage of definitive knockdown after 1, 5, and 10 days in *Rhodnius prolixus* after 10, 30, and 60 minutes exposure to glass or sun-dried mud surfaces treated with a 20% Bayer S-1752 W.P. at the rate of 1 gram active ingredient per sq. meter.

Days After Treatment	Glass									Sun-Dried Mud								
	10 Minutes			30 Minutes			60 Minutes			10 Minutes			30 Minutes			60 Minutes		
	1	5	10	1	5	10	1	5	10	1	5	10	1	5	10	1	5	10
1	90	100	100	100	100	100	100	100	100	0			9	0		50		
5	100	100	100	100	100	100	100	100	100	10			0			0		
12	100	100	100							0	0		0	20		0	40	
35	100	100	100										0	0	0	10	20	30
42	40	100	100										0	20	30	0	30	30
56	100	100	100										0	0	0	0	10	50
63	+)0	0	0										0	10	30	0	30	40
70	+)0	0	20										0	0	50	0	40	100
77	70	100	100	0[a]	20	30							0	20	20	0	20	50
91	100	100	100	0[a]	30	20							0	50	50	0	40	60
99	0	70	90	0[a]	10	0							0	10	10	0	10	10
112	10	80	80	0[a]	10	30							0	0	10	0	10	10

[a] Much used glass plates.

centages of knockdown found in groups of *Rhodnius prolixus* after exposure of 1, 5 and 10 days. The table indicates also the satisfactory persistence of the effects on glass even with a contact of only 10 minutes; 100% knockdown resulted after 91 days of treatment. On the other hand, on adobe surfaces the erratic results obtained show much less persistence, even with exposures of 60 minutes. As in the fly tests, we observed a sudden drop in effect on glass plates after 63 and 70 days, owing to previous use of the plates.

The tests were continued with new plates for the 10-minute exposures, the results confirming the aforementioned.

Table 4.—Number of female *Culex p. quinquefasciatus*, laboratory strain, and mortality percentages[a] 24 hours after exposure to glass or lime-coated sun-dried mud surfaces, treated previously with suspensions of Bayer S-1752 at the rates of 1 or 3 gm. of active ingredient per sq. meter.

Days After Treatment	Glass Plates, 1 Gm./M.²				Sun-Dried Mud Lime-Coated, 3 Gm./M.²	
	5 Minutes		15 Minutes		60 Minutes	
	No. Insects	Mortality (%)	No. Insects	Mortality (%)	No. Insects	Mortality (%)
106					40	12.5
118					42	45.6
139					33	34.2
155	47	41.4	41	71.5		
167	43	44.6	34	20.2		
188	37	28.9	37	19.7		

[a] Mortality percentages calculated according to Abbott's formula. (Jour. Econ. Ent. 18(2): 265–7. 1925.)

Tests with mosquitoes.—The tests summarized in table 4 were made to establish the residual effect of S-1752 on *Culex p. quinquefasciatus* at the end of the observation periods. We observed the same persistent insecticidal effect on glass plates, which produced 41.4% and 71.5% mortality with exposures of 5 and 15 minutes, respectively, after 155 days. The results on adobe and encalado were erratic and showed the active ingredient was less persistent, with mortality rates below 50%, for 1 hour's exposure, 106 to 139 days after the treatment, using a dosage of 3 gm. per square meter.

Biological Control—Past, Present and Future

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NOTE: This paper and the two which follow it were presented as a part of a symposium entitled "The Future Outlook in Biological Control" (Dr. C. B. Huffaker, Albany, California, Moderator) at the Annual Meeting of Section C, Subsection a, held at Salt Lake City, Utah, December 1, 1958. Two additional papers were presented on this program but were not offered for publication: 1) "Utilization of Insect Diseases" by Y. Tanada because it was covered in essential part in the Annual Review of Entomology 4: 279–302, and 2) "Greater Use of Biological Control—General Obstacles, How to Meet Them, New Approaches" by P. DeBach and R. van den Bosch who treated a number of general matters that overlapped to an extent with the previous papers. EDITOR.

ABSTRACT

Speedy and successful control of a number of insects and weed pests, which had spread into areas other than their original habitats, by biological methods may suggest that "the cream has been skimmed off" in the field of biological control. However, in many instances only comparatively superficial studies have been made and the most obvious and apparently promising natural enemies have been selected as biological control agents.

The advent of increasingly frequent and speedy air services to wider and wider areas has opened up more possibilities for the use of the biological control method. Increasing rigidity of quarantine laws and inspection services have not prevented the

accidental introduction of exotic insects and weeds into new areas. Increased trade, and particularly facility and speed of air transport, have more than offset the effects of more stringent quarantine measures in this regard. The spread of pest species into new areas will continue and afford additional opportunity for the use of biological control methods.

The ecological approach to pest problems is suggested in which an insect pest or its complex of natural enemies should not be considered an isolated entity but in relation to the whole environment, keeping in mind the seasonal changes that occur.

Great expansion is indicated in the use of micro-organisms of various types in the control of pests. It is concluded that there is no justification now for considering the method of biological control of decreasing value but that there will be a continued and probably increasing place for this method of control in the future.

It has been suggested that in the field of biological control the "cream has already been skimmed off," and this, as far as possible, was true when such work started. At the time of the first serious efforts at biological control a number of insects and weed pests had been spread into areas other than their original habitats, and some of these afforded promise of speedy and successful control by biological methods. Naturally, these problems were attacked first, and in this regard one can say that the cream of the work was skimmed off—the most spectacular of these early attempts was the introduction into California in 1889 of *Rodolia cardinalis* (Muls.) from Australia to control *Icerya purchasi* Mask.

It might also be stated that with many individual problems tackled from the point of view of biological control, comparatively superficial studies have been made, and the most obvious and apparently promising natural enemies have been selected as biological control agents. Such agents are in many cases not necessarily those that are in fact the key species keeping the pest at a low level in certain areas of its distribution.

On the other hand, the answer to the original suggestion is, also quite as categorically, that the cream has *not* been skimmed off. These two statements are not as contradictory as may at first appear. To carry the analogy a little further, it is natural that, in dealing with a quantity of milk, the cream might be skimmed off first as far as that was possible. However, if we assume that the supply of fresh milk is being continuously replenished, then additional cream will always be present even after a number of skimmings. This is about what has happened with regard to the problem of insect and weed pests and their control by biological methods.

EFFECT OF SPEEDY AIR SERVICE.—Although, as previously said, at the beginning of biological control work those problems promising speedy and successful solution were tackled first, it should be remembered that difficulties of transport made many of these transfers of beneficial insects very difficult if not impossible. The advent of increasingly frequent and speedy air services to wider areas has, even in itself, opened up more possibilities for biological control. As an example of these difficulties might be cited the shipment of Coccinellids from Trinidad, B.W.I., to Fiji against the coconut scale, *Aspidiotus destructor* Sign., when a shipment left Trinidad on January 28th, 1928 and arrived in Fiji on March 6th, the Coccinellids being kept on scale-infested palm trees in cages for the 5-week voyage (Taylor 1935). In contrast, the same species were shipped to Principe, Portuguese West Africa in August 1955 in a minimum of 4 days (Castel-branco 1956).

Successful control of the citrus blackfly, *Aleurocanthus woglumi* Ashby, has been obtained in the Seychelles by means of the parasite *Eretmocerus serius* Silv. brought from Jamaica in 1955. This would have been virtually impossible before air transport was available.

ACCIDENTAL INTRODUCTIONS AND SPECTACULAR RESULTS.—Most of these early instances of biological control involved the transfer of natural enemies of insect pests which had become established in other parts of the world. Although there are some instances of problems involving the use of parasites and predators of related rather than identical species, this type of work was only expanded later and is a further line along which biological control work can develop. It must not be forgotten, too, that with the successes of the early days there were also many introductions into certain areas without due consideration, which turned out to be valueless or even harmful in the long run. The introduction of the mongoose into some of the West Indies might be quoted as an example.

With increasing rigidity of quarantine laws and inspection services it might be thought that the accidental introduction of exotic insects and weeds into new areas would now virtually have stopped. This is far from being the case. Each year many new records of introduced species, some of them potential pests, are made from many areas. Increased trade, and particularly facility of air transport, have more than offset the effects of more stringent quarantine measures. Examples of these are legion, but from the particular point of biological control might be cited the introduction into Hawaii during World War II, along with many other species, of *Dacus dorsalis* Hendel, the catastrophic introduction into Bermuda of the cedar scale, *Carulaspis visci* Shrank., in 1941, *Pulvinaria psidii* Mask., the green shield scale, into the same island in about 1954, *Therioaphis maculata* (Buckton) the spotted alfalfa aphid, into California in 1954, *Ceratitis capitata* (Wied.), the Mediterranean fruit fly, into Florida, 1955, *Anthonomus grandis* Boh., the boll weevil, into Venezuela in 1949, *Lithocolletis messaniella* Zell. into New Zealand in about 1950, *Hyphantria cunea* (Drury) into Hungary from North America in 1940, *Swammerdamia* spp. and *Cnephasia virauregana* from Europe into Canada more recently. As examples of spectacular results obtained using biological control on recently introduced pests might be mentioned again four examples quoted above, first that of *Aspidiotus destructor* introduced into Principe in 1952, biological control initiated in 1955, virtually complete control in 1958; second, citrus blackfly was recorded for the first time in the Seychelles in 1954, biological control undertaken 1955, control 1956–57; and with the spotted alfalfa aphid in California control has been greatly aided since 1954 by a combination of native predators and parasites introduced from the Middle East, and with *Lithocolletis* in New Zealand control is now being obtained with *Apanteles circumscriptus* Nees imported from Europe. It is interesting to note, in spite of quarantine precautions, that in 1957 *Aspidiotus destructor* was recorded for the first time in Jamaica, one of the few tropical areas it had not previously

reached; biological control measures have now been started.

ADDITIONAL OPPORTUNITY FOR USE OF BIOLOGICAL CONTROL.—It is certain that this spread of pest species into other areas will continue, affording additional opportunity for the use of biological control. The alteration of cultivation methods and the development of large areas of monoculture or of few crops, particularly in rapidly expanding tropical agricultural areas, afforded possibilities of development of large populations of pests already existing, endemic or introduced. Such developments, and particularly large irrigation projects where microclimates are considerably altered, will undoubtedly entail entomological problems some of which will be amenable to the biological method.

In the same way, the production of new varieties of various economic plants giving better quality products or higher yields may produce increased insect attack. For example, the attack of borers, *Diatraea* spp., on some new varieties of sugarcane is heavier than on some of the older standard varieties. In other cases damage by an insect species may now be recognized as more serious than it was previously considered.

In these ways additional problems of insect, and weed, control will continue to arise, some of which will give promise of the successful use of biological control. In this respect there will be a continual need for biological control investigations and the introduction of beneficial species.

Weeds probably offer a wide, comparatively unexplored, field for biological control work. In addition to the classic examples of success with cactus control in Australia and South Africa, *Clidemia hirta* in Fiji, *Lantana camara* in Fiji and elsewhere, and more recently those of *Hypericum perforatum* in Australia and Western North America, and *Cordia macrostachya* in Mauritius, there are many more weed pests where detailed investigations may result in successful biological control.

EFFECT OF CHEMICAL METHODS.—It is being increasingly realized that the continuous use of chemical methods in controlling insect and weed pests entails a number of deleterious side effects—quite apart from the accumulation of possibly harmful chemicals in soil and plants. These side effects are complex and in many cases little understood, since the actual causes of the effects observed are often not as simple as at first thought. In the first place, very often when a chemical control program of a given insect pest was undertaken little attention was paid to the effect that the insecticides would have on the natural enemies of the pest in question, or particularly of other insects on the same plants. The effects of chemical insecticides used in orchard sprays on populations of mites (see for example the work of Pickett (1953) in Nova Scotia and of Huffaker & Kennett (1956) in California) and of scale insects on citrus, indicated clearly that consideration should be given to the effect of chemical insecticides on natural control, and on the general ecology of all the insects associated with the crop. In the second place, in many cases it is now known that strains of insects resistant to groups of insecticides are developing following continued application of chemical insecticides.

I am not suggesting that chemical methods of control are not of major importance, but it becomes more obvious as time goes on that the value of these highly effective chemical insecticides is not as unlimited as might at first have been supposed.

The former of the two effects mentioned above indicates that, as is being done with scale insect problems on citrus in California, detailed investigations of the natural enemies of a pest, and the control they exert, should be made before widespread use is made of chemical methods. If possible these latter should be correlated with the degree of natural or biological control existing in individual areas.

This type of integrated program also covers that of control which is carried out by means of the artificial multiplication of native or established parasites and predators for liberation at a time when they are at a low population level in the field, *e.g.*, *Cryptolaemus montrouzieri* in California and Russia, *Diatraea* parasites in the West Indies, Louisiana, and Florida, and *Trichogramma* against both *Diatraea* and other borers in sugarcane, and against several orchard crops in, *e.g.*, the U.S.A. and Russia. This latter type of biological control has at various times been criticized and in many cases, *e.g.*, with *Diatraea* in certain areas, it seems certain that the claims made for successful control were unfounded. More detailed investigation is needed in this sphere.

With regard to the second point, the development of resistance, this is resulting in an increasing interest in the possibilities of biological control of several pests, even those where the chances of successful results seem rather remote. One might cite the example of the planthoppers, *Aeneolamia* spp., in Central and South America causing serious damage to sugarcane and pasture grasses. With developing resistance, chemical control is becoming increasingly expensive and less effective, and biological control investigations are being considered. The codling moth, *Carpocapsa pomonella* (L.), is another case in point. Thus, even where chemical insecticides are used very effectively at present, it seems that biological control methods may still have a place in the future.

AN ECOLOGICAL APPROACH.—It is interesting to note in this connection that an ecological approach to pest problems is developing increasingly. Rather than considering an insect pest or its complex of natural enemies as an isolated entity it is obvious that both should be considered in relation to their whole environment, keeping in mind the seasonal changes that occur.

As simple examples of such an approach might be cited the mass liberations mentioned above of beneficial insects at critical times in the host population cycles. Another simple ecological approach is that of providing suitable plants for the feeding of adult parasites, *e.g.*, *Umbelliferae* planted in Russian orchards, *Cordia* planted along the sides of Mauritius sugarcane fields to attract *Tiphia* spp., Scoliid parasites of the imported white grub, *Clemora smithii* (Arr.). Modifications of agricultural practice which might favor increased natural control (Telenga 1958) are also in this category, and the interareal transfer of natural enemies from areas of declining pest populations to those of increasing population as is being practised to a certain extent at present in Russia, with, it is claimed, some success.

Thus, in those biological control investigations whose object is the selection for introduction into a new area of the most effective controlling agents against a pest, it is

insufficient to study simply the relationship between the natural enemy species and the host-pest. They must be studied in relation to the environment as a whole, alternate host, seasonal fluctuation in populations, et cetera. This idea is discussed in a recent paper by Mesnil (1958), which, I think, is most important in indicating additional possibilities for the method of biological control. The concept that specific parasites and predators are in general of prime importance in controlling insect numbers may need to be reconsidered in the light of recent ecological studies. It becomes increasingly obvious that in biological control work in general, and particularly in that concerned with forest pests (Balch 1958) and others of mixed communities, *e.g.*, sugarcane and wild grass species, detailed ecological investigations are necessary if the best use is to be made of the biological control agents at our disposal (see Tooke (1942) on the successful control of the Eucalyptus snout beetle, *Gonipterus ?scutellatus* in South Africa). This idea is not new, but greater attention has been paid to it recently, and the results are promising.

In the past a number of biological control projects have been abandoned as failures after the unsuccessful introductions of several exotic natural enemies of the pests in question, before any ecological studies have been carried out. Probably, in some of these, more detailed studies of these pests in their areas of origin might provide the answer to successful biological control. The results of the lack of such knowledge are shown in the difference between the success of *Eretmocerus serius* in controlling the citrus blackfly in Cuba and Jamaica, and its complete failure in ecologically different areas in Western Mexico, and possibly also in the failure of introduced Coccinellids to control several scale insects in Bermuda under apparently very favorable conditions. Here the influence of ants and possibly of predatory lizards has been investigated recently (Simmonds 1956).

USE OF MICRO-ORGANISMS.—In the past few years there has also been a very great expansion of interest in the utilization of micro-organisms of various types in the biological control of pests. With the specialized knowledge and techniques required this must become a distinct branch of insect control, but it should be remembered that this is still biological control, and that the effects of diseases on both the pest and its other natural enemies will always have to be considered as a whole. While disease studies will inevitably be carried out separately there should be close cooperation between workers in this field and ecologists and those working on other aspects of biological control.

We also should mention the recent developments in Russia of the use of combined sprays containing insecticides and micro-organisms which appear to give very much greater control than either used alone (Telenga 1958).

FUTURE OF BIOLOGICAL CONTROL METHODS.—In the various ways mentioned above there are possibilities for a wide expansion in the utilization of biological control methods in the future. This does not mean that it is a panacea for all problems on the control of insects, weeds, and other pests. It is not. The proportion of successes, partial successes and failures in future work will probably remain about the same as in the past, and spectacular successes will be few. It is hoped, however, that with increasing amounts of data on different types of problems available, particularly of an ecological nature, the assessment of the chances of success with individual problems will improve.

There is therefore no justification for considering that the method of biological control is now of decreasing value as contended by Taylor (1955). I have already discussed this paper elsewhere (Simmonds 1956), but I think that the general contention that there will be a continued and probably increasing place for biological control methods in the future is clearly indicated. This is also suggested from a rather different standpoint in a recent book by Elton (1959), "The Ecology of Invasions by Animals and Plants," in which he stresses the ever-increasing trend for many species to become more widespread within the limits of their climatic tolerance, and the explosive development of populations which may follow their introduction without normal natural enemies into new areas. This book gives the impression that, far from the cream having been skimmed off the general field of biological control work, we have hardly made a beginning.

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Utilization of Native Parasites and Predators[1,2]

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Abstract

In apple orchards in Nova Scotia, general use of a program of selective sprays for several years has been followed by a reduction in the amount of damage caused by important insect and mite pests. These pests include the oystershell scale (*Lepidosaphes ulmi* (L.)), the codling moth (*Carpocapsa pomonella* (L.)), the eye-spotted bud moth (*Spilonota ocellana* (D. & S.)), the apple aphid (*Aphis pomi* DeG.), the rosy apple aphid (*Anuraphis roseus* Baker), and the European red mite (*Panonychus ulmi* (Koch)). Although proof has not been obtained in all cases, the evidence indicates that, when selective pesticides were applied, beneficial species survived in greater numbers, and in some cases increased substantially. In developing such a program emphasis should be placed on use of selective pesticides, timing of pesticide applications to avoid susceptible stages of beneficial species, and establishment of the minimum dosages required to control pests.

Because satisfactory reviews are available I do not propose to refer here to the extensive literature on this subject. I shall instead review the work done in Nova Scotia. In accordance with the nature of the investigations I shall refer to parasites and predators generally *in toto* and in only a few cases to individual species. Furthermore, I shall not attempt to distinguish between indigenous species and those accidentally introduced.

Parasites and predators are favored by conditions suitable for their development and reproduction. The physical environment must be adequate; food must be available; and the life cycles of the entomophagous species and their hosts must be synchronized (Baker & Jones 1934, Smith & DeBach 1953).

The Use of Selective Pesticides.—In 1943, when we began investigating the long-term influence of pesticides on the population trends of apple insects, four major pests were known: the oystershell scale (*Lepidosaphes ulmi* (L.)) the European red mite (*Panonychus ulmi* (Koch)) the eye-spotted bud moth (*Spilonota ocellana* (D. & S.)) and the codling moth (*Carpocapsa pomonella* (L.)). At that time dormant oils, the dinitro compounds, bordeaux, sulphur, lead arsenate, and nicotine sulphate were the pesticides in general use. When the oils and sulphur were discontinued and ferbam was substituted for the latter, suitable conditions were provided for increases in the populations of the parasite *Aphytis mytilaspidis* (LeB.) and the predaceous mite *Hemisarcoptes malus* (Shimer), which reduced the scale population to insignificance throughout the Annapolis Valley within 2 years (Lord 1947). Although this scale may be found in almost any apple orchard in Nova Scotia, the population has not attained outbreak proportions in the last 10 years except where sulphur fungicides have been used several times a year for 3 or more consecutive years.

In Nova Scotia and elsewhere serious outbreaks of phytophagous mites are largely man-made phenomena. Dormant-type oils, the dinitro compounds, sulphur, and many of the synthetic organic pesticides kill most predators of mites. When our investigations started, the majority of orchards in the Annapolis Valley had substantial mite populations. Now, with the more or less general adoption of a schedule of selective sprays, high populations of phytophagous mites are uncommon and occur only in orchards treated with one or more of the abovementioned pesticides. Many of our growers have not used acaricides for the past 5 years and the foliage on their trees retains a normal healthy color and texture throughout the growing season in marked contrast to those orchards where nonselective insecticides and fungicides are used without effective acaricides.

For many years the eye-spotted bud moth was the most serious pest of apples in Nova Scotia. Although it has not been possible to determine the role of each of the many factors that reduced this species to minor pest status, it is apparent that an increase in natural control agents following the general use of selective pesticides has been responsible to a marked degree. The braconid parasite *Agathis laticinctus* (Cresson) increased substantially in numbers after sulphur and lead arsenate were discontinued, accounting for as much as 90% of the overwintering larvae (Stultz, Kentville (unpublished)). Furthermore, there is evidence that several general predators, *i.e.*, some of the anthocorids, mirids, coccinellids, pentatomids, thrips, and the predaceous mite *Anystis agilis* Banks, are effective against this pest, especially at low-prey densities.

Of the four major pests mentioned above, the codling moth was the last to reach that status, the population increasing gradually for several years until it reached a peak in 1948. Similarly, it has decreased in importance more slowly than the others. During the years it was increasing, lead arsenate was generally used for its control until 1949 when there was a shift to DDT and parathion; these were used widely until 1953, when ryania came into general use.

Since 1948 an annual record of damage of codling moth to apples has been taken in approximately 80 orchards at random throughout the Annapolis Valley. Although variation has been observed from year to year, usually attributable to the number of fruits per tree, the codling moth damage has declined as shown by the figures for total injuries per 100 fruits:

1948	*1949*	*1950*	*1951*	*1952*	*1953*	*1954*	*1955*	*1956*	*1957*	*1958*
30.8	8.1	11.2	13.9	13.4	14.9	5.5	6.5	6.9	3.3	4.1

Furthermore, it should be pointed out that much more spraying for codling moth control was done in the early part of the period than later. A variety of spray schedules were used in the orchards sampled, ranging from a few that received two or more applications of DDT each year to others not treated specifically for the codling moth.

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[2] Presented as an invitational paper of Section C at the Sixth Annual Meeting of the Entomological Society of America, held at Salt Lake City, Utah, December 1 to 4, 1958. This paper was endorsed and communicated for publication by Carl B. Huffaker.

Figure 1 shows the results in a typical orchard when insecticides were discontinued in 1950 after 2 years' treatments with DDT and parathion. A 10-acre block of a somewhat isolated orchard was divided into two equal plots. One-half was treated with six or seven applications of the fungicide glyodin and the other half with two or three early bordeaux sprays followed by three glyodin applications just before and after bloom, and the schedule was completed with a single light bordeaux application. In 1954 the treatments were switched on the plots to determine if the position of each plot had a bearing on the results. The low damage in 1950 was probably due to the highly effective spray program in 1949, which reduced the codling moth population to almost zero. In 1951 the population increased substantially, either by multiplication in the orchard or by an invasion of moths from orchards some distance away. By 1952 the beneficial species had become re-established to a point where they provided considerable control. The poorer control on the bordeaux-glyodin plot is thought to be due to higher deposits of inert residues, which, according to Bartlett (1951) and other investigators, reduce the effectiveness of parasites and predators. As codling moth injury decreased in both plots less difference was evident in control between the two treatments, and since those in both are now below the average for the 80 orchards it is probable that the maximum level of natural control has been reached under the present conditions and any further improvement will require the introduction of artificial methods.

The following figures show the average injuries by the codling moth to apples in a 5-acre orchard after treatments with three insecticides during a 5-year period:

Year	*Insecticide*	*Stings per 100 Apples*	*Deep Entries per 100 Apples*
1950	DDT	1.0	1.0
1951	None	5.0	38.0
1952	Lead arsenate	21.0	32.0
1953	Ryania	7.0	0.2
1954	None	0.4	1.4

The main point of interest in this table is the amount of damage by the codling moth when DDT was discontinued, in contrast with that in the year after ryania was used, and the ineffectiveness of lead arsenate in this orchard.

An explanation of the difference between ryania and DDT is suggested by the numbers of several beneficial groups taken by fumigating whole trees that had received three successive sprays of the respective insecticides:

Treatment	*DDT*	*Ryania*
Predaceous thrips	9	293
Miridae	3	151
Coccinellidae	0	9
Pentatomidae	1	14
Hymenoptera	41	252
Arachnida	15	98

For many years the apple aphid, *Aphis pomi* DeG., and the rosy apple aphid, *Anuraphis roseus* Baker, were frequently highly destructive in Nova Scotia. Although no detailed studies have been made on these pests it appears to be more than a coincidence that they have become progressively less injurious during the last decade, and I know of no orchard being treated with a selective spray program where either of these insects has been of more than minor importance during the last 5 years. This fact appears to be in marked contrast with the situation in many apple-growing areas where the apple aphid particularly has increased in importance.

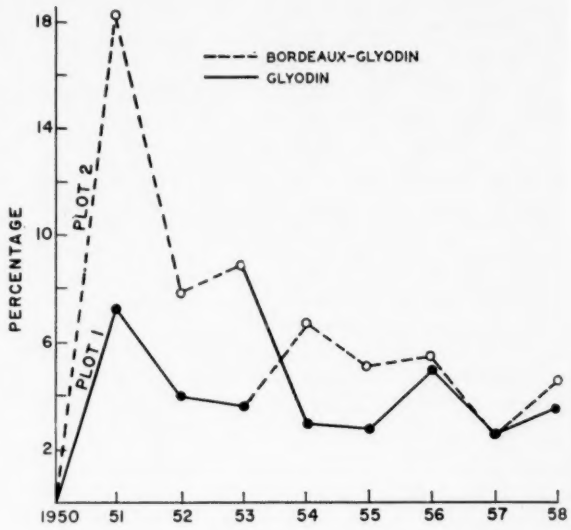

FIG. 1.—Percentages of apples injured by the codling moth in adjoining 5-acre plots of somewhat isolated orchard when sprayed with slightly different fungicide programs after discontinuing insecticides. The treatments were reversed in 1954.

THE USE OF MINIMUM DOSAGES AND THE TIMING OF APPLICATIONS.—In addition to the policy of choosing selective pesticides, some progress has been made by reducing the dosage of some of the more toxic ones to a point where a pest species is controlled and beneficial species are harmed little. In Nova Scotia the fall canker-worm (*Alsophila pometaria* (Harr.)), the winter moth (*Operophtera brumata* (L.)), and the green fruitworms (*Lithophane* and *Xylena* spp.), may be satisfactorily controlled with as little as 1 ounce of 50% DDT wettable powder per 100 gallons, a dosage that has little effect on most predaceous species at the time of year it is used.

The timing of applications of pesticides is sometimes important. In Nova Scotia preblossom applications of lead arsenate interfere with the development of *Agathis laticinctus*, a parasite of the eye-spotted bud moth, whereas postblossom applications seem not to be detrimental (Stultz, Kentville (unpublished)). Applications of nicotine sulphate in the delayed dormant or prepink stages of apple-bud development for the control of aphids or the apple sucker do not seriously reduce populations of predaceous mirids or anthocorids, probably because most of them are still in the egg stage (Sanford, Kentville (unpublished)).

GENERAL CONSIDERATIONS.—The longer the Nova Scotia studies continue the more evident it becomes that many factors are involved in biological balances, and the role of each is difficult to evaluate. More and more we are giving increased attention to general predators, for if these are to be maintained in effective numbers, adequate food must be available either in the orchard itself

or in the environment. In some cases it may not be highly detrimental to destroy some of the beneficial species if, nearby, adequate numbers exist of the same or other effective species sufficiently mobile to replace quickly those destroyed. Consequently, we may have to consider not only the immediate area or crop concerned but the whole environment, including large areas and a diversity of crops as well as uncultivated areas, bushland and so forth. For instance in Nova Scotia MacLellan (1958) points out that native woodpeckers are more effective in destroying overwintering codling moth larvae on apple trees in areas where there is ample bird cover than where such cover is inadequate. In further studies MacLellan (unpublished) has found that in a 4-square mile area with adequate cover 23 woodpeckers were noted compared with 10 in an area of similar size where cover was sparse.

In Nova Scotia as more and more orchardists have adopted the modified control program the pest situation has gradually improved. In England some concern has been expressed regarding the tendency to destroy, in modern machine farming, hedges, headlands, and the woody vegetation on roadsides (Way *et al.* 1956). Such vegetation acts as preserves and provides cover for beneficial as well as some harmful species. Considering the balance, the former may outweigh the latter; at least, the situation should be studied carefully before irreparable changes are made. Basically, natural control should be more effective under a semipermanent type of agriculture. It takes time to establish biological balances but these may be destroyed very quickly. That marked changes in animal populations are less likely to occur in the tropics than elsewhere appears to be the consensus. This premise undoubtedly implies an environment with a more or less permanent and rich floristic composition wherein the fauna apparently provides its own controlling mechanisms. Where wide seasonal variations in weather or changes in the flora occur within short periods, instability in the fauna is likely to be more evident. Consequently, all these factors must be considered in any attempt to improve the effectiveness of native parasites and predators.

Contrary to popular belief, exceptionally low temperatures or other abnormal weather fluctuations may favor the pest species by destroying or retarding the development of beneficial species (Lord & MacPhee 1953). The establishment of a suitable local environment for beneficial species may be possible where a more or less permanent crop is being grown, but this is frequently impossible with large plantings of an annual or biennial crop.

SOME GENERAL CONCLUSIONS.—Fifteen years of investigations and observations on the long-term influence of spray chemicals on pest populations in Nova Scotia apple orchards lead to the following conclusions:

1. The routine repetitive application of widely toxic pesticides as insurance against outbreaks of arthropod pests frequently creates, through the general destruction of parasites and predators, more serious problems than those corrected.

2. Several apple pests presently of great economic importance would not exceed the economic tolerance if pesticides did not interfere with the activities of biotic control agents.

3. Several years may be required for parasites and predators to reestablish themselves, even under favorable conditions, if they have been eliminated over a wide area.

4. It is usually impractical to attempt to reestablish the biological control of pests, especially highly mobile ones, in relatively small areas.

5. The development of strains resistant to chemical pesticides emphasizes the importance of recognizing the role of biological control agents and focuses attention on the desirability of combining biological and chemical control.

6. More emphasis should be placed on the development of selective pesticides, the timing of pesticide applications to avoid susceptible stages of beneficial species, and the establishment of minimum dosages required to control pests.

7. More precise knowledge of the mechanisms involved in population dynamics is a prerequisite to avoiding the complications inherent in depending entirely on the chemical control of pests.

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Integrated Control Programs in the Future of Biological Control

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ABSTRACT

Integrated control is applied pest control which combines and integrates biological and chemical control. Chemical control is used as necessary and in a manner which is least disruptive to biological control. Integrated control may make use of naturally occurring biological control as well as biological control effected by manipulated or introduced biotic agents. The development of integrated control programs opens new horizons for the utilization of natural and biological controls.

With each increment to the human population, we intensify our competition with the insects for the food resources of the earth. Surpluses of some products in limited parts of the world should not blind us to the facts that three-fourths of the world's people are undernourished and that there is a net gain of about 100,000 people in the world each day (Hertzler 1956). To insure success in our battle with the insects, we must utilize every possible means to combat them—not biological control alone, not chemical control alone. Furthermore, the production and protection of any food or fiber crop must be considered in its entirety and from the long-range point of view (Decker 1958). This is the ecological or total approach to control. It includes the simultaneous consideration of insects, diseases, plant nutrition, plant physiology, and plant resistance, as well as the economics of the crop. It does not depend entirely on biological or cultural controls nor does it exclude chemical control. It utilizes all of them to their fullest to produce effective and economical pest control (Smith & Allen 1954, Pepper 1955).

Today the products from our fields and orchards are of higher quality and in greater quantity than ever before. A major reason for this fact lies in the steady and substantial improvements in insect control. At the same time, we still have serious unsolved problems and, whenever we relax our pressure on the insects, they quickly rebound.

CHEMICAL CONTROL ALONE.—When insect control is developed with a narrow or one-factor approach, the results are frequently inadequate, uneconomical, or temporary. For example, a straight chemical control program is too expensive for many crops with low unit value. Even with those crops where more money is available for control the costs are becoming prohibitive in some cases. Some farmers claim they are on a never-ending upward spiral of increasing insecticidal treatments and costs.

At the same time, where chemical controls are used alone, the insects seem to be fighting back. Pest problems that seemed to be solved a few years back are with us again. Other pests that formerly were insignificant are now serious pests. In addition, the side effects of pesticides on soils, trees, and fruit quality have complicated the chemical control picture.

It is shortsighted to develop an expensive chemical control program for the elimination of one insect pest and ignore the impact, both beneficial and harmful, of that chemical program on other insects. But that policy is no worse than attempts to utilize parasites for one pest without considering the chemical measures that may be necessary on the same crop (Hagen & Smith 1958).

BIOLOGICAL CONTROL ALONE.—A few unrealistic individuals have advocated eliminating the use of all chemicals and go back to nature's way of taking care of the problem. This situation would certainly be ideal, if it would work. Among other benefits, this procedure would remove a great financial burden from the farmer. That such a Utopia cannot be realized should be obvious from two facts:

1) Our agricultural fields and orchards are not works of Mother Nature but are highly artificial, man-made "factories" for producing food and fiber. We have destroyed native forests and grasslands. We have developed special strains of plants and animals and moved them about the earth. We have changed deserts into lush gardens by huge irrigation projects. We have devoted large areas to a single crop. In these and other ways we have altered the balances that once existed (Thomas 1956). In our fields and orchards we have provided an abundant food supply and other environmental conditions highly favorable for insects. The complex of insects in these man-made environments is not the same as before man disturbed the scene. Some that were abundant are now absent or scarce. Some that were scarce are now abundant. Others have been introduced. And the changes continue.

2) Even if we assume that all the insects were regulated and controlled in their native undisturbed conditions, we cannot assume that in our "agricultural factories" such regulations will keep the pests below our *artificial* economic levels.

On the other hand, these facts of life do not mean we should forget natural and biological controls. We cannot go back to the original primitive conditions, but we can take advantage of natural control to establish new balances in our favor.

INTEGRATED CONTROL.—If biological control alone or chemical control alone are inadequate, the development of methods of utilizing the two together would then seem logical. In the past some entomologists have been inclined to reason that the two were incompatible. Today, the evidence is before us that they are not necessarily alternative methods; they may be complementary (Wille 1951, Huffaker & Kennett 1956, De Bach 1958, Pickett *et al.* 1958, Ripper 1958, and others). Parasites, predators, and disease may be manipulated to hold some pests at subeconomic levels all or part of the time. Chemical controls can be used in a manner that does not disrupt the natural control of other pests and at the times when natural control is inadequate. This complementary use of chemical and biological control is called integrated control. It is a comprehensive approach to insect control that makes full use of biological control but also utilizes chemical control where necessary and in a manner that is least disruptive to the biological control.

One of the reasons for the apparent incompatibility of biological and chemical control is the failure to recognize control of insects as a complex problem in ecology. Any attempt to reduce the levels of one kind of insect by chemical treatments, by modification of cultural practices, by introduction of parasites, or by other methods, will also affect other organisms in that same environment. For this reason, all the insects and mites, both beneficial and harmful, and all the control methods must be considered together.

REQUIREMENTS OF INTEGRATED CONTROL.—Integrated control programs are dependent on the presence of natural controls capable of holding the pests below economic levels all or at least part of the time. In most of our fields and orchards, there are potential pests held continually at subeconomic levels by natural regulating forces. Other pests cause intermittent damage, but are held below economic levels by natural controls part of the time. In a single field or at any given time, such intermittent pests may rise to high numbers, while in other fields or at other times they remain low. It is with such types of pests that integrated control is especially valuable. The occasional potentially destructive population is detected before damage occurs and is reduced in a manner that permits the natural controls which prevailed before and which prevail elsewhere to take over again.

In addition to potential pests and intermittent pests, permanent pests are present as problems nearly every year and in nearly every field. Even with this type of pest, natural controls may be significant in reducing the degree of damage. From the standpoint of integrated control, it is important that a program of required chemical control of permanent pests does not disrupt natural control of potential and intermittent pests.

If natural controls have been destroyed or are absent, they must be reestablished or introduced before integrated control can be effective. When partial biological control is present, it can be fostered and utilized by employing chemicals only at the times or places where the biological control is inadequate. It is essential that the chemical controls do not disrupt biological control of other pests or eliminate potential controls for the next crop or season.

To put integrated control into practice, it is necessary to have precise knowledge of both beneficial and harmful insects and mites and the impact or potential impact of the harmful pests on the crop. The first mentioned necessity requires good population sampling methods. The second requires a sound knowledge of economic thresholds.

The sampling methods of research entomologists and biodemographers are usually too time-consuming and tedious to be of practical value in an integrated control program. Special index methods are needed which are rapid and simple to use. Ideally, the methods should be of such nature that the grower can use them without interfering with his other farming responsibilities. In some instances the difficulties and complexities involved in determining the status of pest populations require the services of a qualified supervising entomologist.

Economic thresholds—that is, the lowest population levels capable of causing damage—must be established for each pest species. Such conclusions are not easily reached. In most instances, we are dependent on the educated guesses of experienced field entomologists. Our changing economy, variations in consumer demand, and the difficulties of measuring the effects of insects on yield and quality often make the scientific assessment of economic damage extremely laborious. Nevertheless, reasonable and realistic economic thresholds are necessary before integrated control can be developed to its fullest. Success is dependent on holding insect populations below scientifically established economic levels rather than on attempts to eliminate insects. This means that we should withhold treatments when the populations are subeconomic and use insecticides in a selective manner when the populations are economic or threaten to be economic.

The selective action of insecticides may be produced in three ways. 1) The insecticide itself may be selective in its toxicological action. Narrow-range toxicants may be utilized to reduce a pest of concern and at the same time spare the beneficial forms. A particular material may be selective in one situation and not in another; or it may be selective at low dosages, but not at high dosages. In some instances, a disease pathogen such as a selective insecticide, may be used (Steinhaus 1956). However, pathogens are not necessarily selective between harmful and beneficial insects. 2) We can produce a selective action on a pest-parasite complex by treating only those areas where the pest-parasite ratio is unfavorable. This method is one of the bases of supervised control of the alfalfa caterpillar in California. 3) Proper timing of insecticides can produce a selective action on the pest-parasite complex. Materials which are nonselective toxicologically can sometimes be used in integrated control programs especially if they have no residual action or for only a brief time. In such situations an intimate knowledge of all regulating factors is required.

As a further point against the ideas of eradication, the ideal selective material is not one that eliminates every individual of the pest species and leaves all the beneficial forms. After treatment with such a material, the predators and parasites would be forced to leave or starve. The ideal selective material is one that throws the balance back in favor of the natural enemies. We must remember that some aphids are needed to produce lady beetles, and scale insects to produe *Aphytis*.

The practice of fixed-spray schedules or treatments based on the mere presence of a pest are often incompatible with the principle that it is necessary to leave some pest insects. Over the years we have developed elaborate spray schedules, especially for our tree fruits, including insecticides, acaricides, fungicides, bactericides, and other types of materials aimed at a great variety of pests. It is not possible to avoid all such schedules or materials. In many cases, prophylactic treatments must be applied to guard against frequently recurring pests. Nevertheless, we should attempt to develop methods of predicting when the occasional pests will occur at economic levels. The necessary fixed sprays should be selective treatments.

It is not enough to develop integrated control methods. These methods must be put into practice. The principles and reasons for integrated control must be explained to

the farmers. This is a big but necessary job in education. We all know of good control methods which have been ineffective in reducing the problems because they were unacceptable to the farmer.

Even when our knowledge is sufficient to outline an ideal integrated control program, it may not be possible to put it into practice immediately. The effects of current programs and practices would last for years. Effective natural control will have to be reestablished. This may be very slow and difficult in many cases (De Bach 1951).

INTEGRATED CONTROL OF THE SPOTTED ALFALFA APHID.—The recent development of integrated control for alfalfa insects is an example of how by proper selection, dosage, and timing, chemicals can be used with natural controls to achieve a better control than could be obtained by either method alone (Stern, *et al.* 1959). In most of California, native predators, introduced parasites, and entomogenous fungi now keep the spotted-alfalfa-aphid populations below the economic threshold for most of the years. As utilized today, the integrated control program has two guideposts. *First*, no alfalfa field is ever treated with a pesticide unless population counts in that field clearly demonstrate that the field is threatened with economic damage. This means that during critical times of the year, the aphid populations must be followed carefully. Treatments are timed by the pest population levels and not according to a fixed time schedule. *Second*, when it is necessary to treat an alfalfa field, a selective insecticide is applied at a dosage that gives adequate control, but not necessarily eradication of the spotted alfalfa aphid. Most of the aphid's natural enemies survive the treatment and persist on the remaining aphids.

This integrated control program for the spotted alfalfa aphid has been used successfully throughout California since 1958. By contrast, in former years, when nonselective materials were used, the parasites and predators were largely eliminated. As a result following such treatments, the aphid populations often increased rapidly to economic levels and repeated chemical treatments were necessary to maintain control. In some instances, these repeated treatments hastened the development of insecticide-resistant strains of aphids.

The insect problems of field crops such as alfalfa appear to be particularly suited to the development of an integrated control program. It will be more difficult to establish a successful integrated control program for pests of deciduous fruits (Barnes 1959). Among other factors, the problems of orchards are different from those of field crops because the plantings are relatively permanent and usually a greater variety of pests are present at any one time.

CONCLUSIONS.—Our thoughts have been centered on the problems of agricultural entomology but we feel that the principles should apply as well to other phases of applied entomology. It must be realized, of course, that in those control problems where the economic threshold is extremely low, it is unlikely that natural controls will be adequate and in some cases may not even be helpful. The levels at which insect vectors must be held to prevent the spread of diseases is such an example.

The development of integrated control programs opens new horizons for the utilization of biological control. It is a goal to be achieved and to us appears necessary in our battle with the insects. It will come about by the cooperative effort of research groups, the extension services, industry and farmers.

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Biological and Control Studies on *Estigmene acrea* (Drury), a Pest of Corn in the Yaqui Valley, Sonora, Mexico.[1]

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ABSTRACT

In the Yaqui Valley of Sonora in northwest Mexico, the salt-marsh caterpillar, *Estigmene acrea* (Drury), has caused severe damage and stand reduction to fall-planted corn during 1957 and 1958. Field observations and insectary studies indicate that the preferred native host plants both for oviposition and for larval development are Amaranth, *Amaranthus palmeri* S. Wats., and ground cherry, *Physalis angulata* L. Studies of the biology of *E. acrea* indicated that the time required for development from egg to adult is approximately 1 month during July, August, and September, when the largest populations are produced, and that four or five generations occur per year. Natural enemies of *E. acrea* observed in this region include a Coccinellid and a Malachiid attacking the eggs, and two species of Reduviids and a fungus attacking the larvae. In chemical control tests, Dipterex® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate) at 0.5 kg./ha. has shown promise. Toxaphene 2.9 kg., endrin 0.4 kg., ethyl parathion 0.5 kg., methyl parathion 0.5 kg., Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) 0.5 kg., and heptachlor 1.0 kg. were not effective.

In the Yaqui Valley of Sonora, Mexico, farmers recently have become interested in expanding their rotations to include several new crops. One of the more important is corn, the best yields of which can be expected with late varieties planted in the fall (August 15 to September 1).

In the fall plantings of 1957 and 1958 severe damage resulted from the feeding of large populations of the salt-marsh caterpillar, *Estigmene acrea* (Drury).[4] Larvae from cotton and native weed hosts migrated into adjacent corn fields, where as many as 13 larvae per plant have been observed on young plants 5 to 20 days old. Midveins of leaves are not usually consumed, but when small plants are attacked they may be completely destroyed. Once this damage has reduced corn stands, it is usually too late to replant, since a crop will not be produced before normal frost season. When plants are not destroyed it is estimated that yield reductions of 1 or 2 tons per hectare may result from damage to the foliage.

Because of this problem's importance, studies were initiated at the Northwestern Research Center, Mexican Ministry of Agriculture near Ciudad Obregón, Sonora, in the fall of 1958, directed toward the development of appropriate control measures for this species on fall-planted corn. This paper summarizes the first season's results from field biological observations, insectary studies, and preliminary field chemical control tests.

BIOLOGICAL OBSERVATIONS.—Although adult moths of *E. acrea* were taken in the light trap throughout the year at Ciudad Obregón, this species is most active and reproduces most rapidly during July, August, and September. Large numbers of individuals pass the colder months from November to February in the pupal stage, hidden in ground trash in the fields or along irrigation canals and drains. The moths from these pupae emerge in the spring and deposit their eggs on cotton and on several native weeds where the larvae develop. Amaranth, *Amaranthus palmeri* S. Wats., is the preferred native host. Others, in order of preference, include ground cherry, *Physalis angulata* L., angle-pod, *Gonolobus* sp., and malva, *Anoda pentaschista* Gray.

At the height of the reproductive season, July and August, masses of 1,000 to 1,500 eggs have been observed on *A. palmeri*, usually on the under side of the leaves. These cream-colored eggs, slightly less than 1 mm. in diameter, turn dark gray-blue shortly before the larvae emerge. The small larvae feed on the leaf near the site of the egg mass for 5 or 6 days, then migrate to other parts of the plant and to other plants.

After about 17 to 19 days of feeding the pilose larvae form a cell of body hairs within which they pupate. Ten to twelve days later the adults emerge. In the region of Ciudad Obregón there are probably four or five generations of *E. acrea* each year.

LABORATORY STUDIES.—The biology of *E. acrea* was studied in the insectary on *A. palmeri* and the two most important cultivated hosts: corn, *Zea mays* (variety San Juan), and cotton, *Gossypium hirsutum* (variety Delta Pine 15). One complete cycle from egg to adult stage was reared in small vials (3 cm. in diameter by 7 cm. high). Ten individuals were allowed to develop on each of the three hosts. Observations were recorded every 24 hours, and care was taken to have fresh leaf material available at all times for the larvae. Temperature records taken during the study indicated an average maximum of 37.3° C. and an average minimum of 25.5° C. The average time required for the 10 individuals to complete each stage of development on the different hosts is presented in table 1. In general, very little variation was noted among individuals on the same host. Development was retarded 2 days in the sixth instar and pupal stages on cotton and corn in comparison with *A. palmeri*. The time required for development determined in this study (30 to 34 days) is slightly greater than the 21 days reported by Telford

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[4] Identification confirmed by Dr. W. D. Field, Smithsonian Institution, Washington, D. C. Other insect identifications were made as follows: *Coleomegilla maculata* by Dr. E. A. Chapin, Harvard University, Cambridge, Massachusetts; *Collops femoratus* by M. Y. Marshall, M. D., Veterans Hospital, Murfreesboro, Tennessee; and *Zelus laericollis* by Dr. P. D. Ashlock, Insect Identification and Parasite Introduction Laboratories, U. S. Department of Agriculture. The fungus *Entomophthora aulicae* was identified by Dr. Irvin M. Hall, Department of Biological Control, University of California, Riverside. Host plant identifications were made by Mr. Thomas Soderstrom, Yale University, and Ing. B. Hernández Xolocotzi, National School of Agriculture, Chapingo, Mexico.

Table 1.—Average number of days required for developmental stages of *Estigmene acrea* on three different host plants in the insectary. Ciudad Obregón, Sonora, Mexico. June 15–July 19, 1958.

Stage of Development	Host		
	Cotton	Corn	*A. palmeri*
	Days	Days	Days
Egg	3	3	3
1st instar	2	2	2
2nd instar	2	2	2
3rd instar	2	2	2
4th instar	2	2	2
5th instar	3	3	3
6th instar	8	8	6
Pupa	12	12	10
Total	34	34	30

(1957) from Arizona and less than the 45 days reported by Stracener (1931) from Louisiana. There is closer agreement with the results of Hinds (1904) from Texas.

To investigate the influence of host plants on larval development further, a second study was conducted in the insectary, including the hosts used in the biological study and in addition the three other native hosts, *P. angulata*, *Gonolobus* sp., and *A. pentaschista* Gray. Fifty larvae were allowed to emerge from a large field-collected egg mass and 10 individuals were reared on fresh foliage from each of the five hosts. Individual larval weights were taken after 10 days and mortality was recorded after 15 days. No mortality was observed at the end of the 10-day period. Weight data were studied statistically by analysis of variance. The results, presented in table 2, clearly indicate that *A. palmeri*, *P. angulata*, and *Gonolobus* sp. are more suitable hosts than either cotton or corn for the salt-marsh caterpillar.

NATURAL CONTROL FACTORS.—In the field several predaceous insects have been observed attacking *E. acrea*. The Coccinellid *Coleomegilla maculata* (De Geer) and the Malachiid *Collops femoratus* Schffr. consume the eggs. Two species of Reduviids, *Zelus laevicollis* Champ. and *Sinea confusa* Caud., attack young larvae. Late in the season of 1958 many larvae were killed by the fungus *Entomophthora aulicae*. None of these natural enemies appear to be sufficiently effective to prevent the development of large populations of the salt-marsh caterpillar during late summer and fall. Effectiveness of predators in the area may be reduced by the application of insecticides in cotton.

Table 2.—Average weight of larvae of *Estigmene acrea* after 10 days feeding on foliage of five different host plants and per cent mortality occurring after 15 days on the same hosts. Ciudad Obregón, Sonora, Mexico. July, 1958.

Host	Average Weight After 10 Days[a] (Mg.)	Mortality After 15 Days (%)
A. palmeri	82.3	0
P. angulata	66.6	70
Gonolobus sp.	55.8	30
Corn	39.2	70
Cotton	34.2	60
A. pentaschista	18.4	10

[a] Average weights not connected by the same vertical line are significantly different from each other at the 5% level of probability according to Duncan's (1955) Multiple Range Test.

CHEMICAL CONTROL.—In the fall of 1958 two tests were conducted with several insecticides applied directly as a spray with small hand sprayers to infested corn plants.

The first of these tests was applied on September 5 to a small plot of 5-day-old corn. The insecticides used were Dipterex®, (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate), ethyl parathion, and Thiodan® (6,7,8,9,10,10 - hexachloro - 1,5,5*a*,6,9,9*a* - hexahydro - 6,9-methano-2,4,3-benzodioxathiepin-3-oxide) at 0.5, 0.5, and 0.2 kilograms of pure material, respectively, in approximately 200 liters of water per hectare.[5] The Dipterex used was a soluble powder containing 50% of the active ingredient and the other two insecticides were emulsion formulations. Each plot consisted of two rows 10 meters in length. All treatments, including the untreated check, were replicated three times. Twenty hours after the application was completed, the dead larvae in each plot were counted. Data taken were subjected to the analysis of variance using a logarithmic transformation. The results are presented in table 3, where the geometric mean number of larvae killed for each treatment and the results of Duncan's Multiple Range Test for significance at the 5% level of probability are shown. Dipterex killed significantly more larvae than parathion, and parathion gave better control than Thiodan. Dead larvae in the untreated check were probably migrants from treated plots.

Table 3.—Relative kill of larvae of *Estigmene acrea* obtained in small corn (4 to 6 inches tall) with three insecticides. Ciudad Obregón, Sonora, Mexico. September 5, 1958.

Insecticide	Dosage[a] (Kg./Ha.)	Mean No. of Larvae Killed[b]
Dipterex	0.5	251.0
Ethyl parathion	.5	109.6
Thiodan	.2	12.7
Check	—	8.6

[a] 1 kg./ha. = 0.89 lb./acre.
[b] Means not connected by the same vertical line are significantly different from each other at the 5% level of probability according to Duncan's Multiple Range Test.

A second test was applied in the field of a cooperating farmer on September 11 in somewhat larger corn, about 1 foot high. Two concentrations of each of seven insecticides were applied with hand sprayers to plots 5 rows by 10 meters in approximately 200 liters of water per hectare. The insecticides and concentrations in kilograms of pure material per hectare used were: Dipterex, 0.25–0.5; toxaphene, 1.4–2.9; endrin, 0.2–0.4; ethyl parathion, 0.25–0.5; methyl parathion, 0.25–0.5; Thiodan, 0.25–0.5; and heptachlor, 0.5–1.0. All formulations were emulsion concentrates except the 50% Dipterex, a soluble powder. Again, treatments were replicated three times. A moderate rainfall occurred after the treatments were applied. Counts were made of live and dead larvae on the ground and on plants in the three central rows of each plot 30 hours later. All dead larvae were on

[5] The Dipterex and Thiodan used in these tests were supplied by Quimicas Unidas, S. A., and Farmacolor, S. A., respectively.

Table 4.—Relative kill of larvae of *Estigmene acrea* obtained in corn (12 to 18 inches tall) with high concentrations of seven insecticides. Ciudad Obregón, Sonora, Mexico. September 11, 1958.

Insecticide	Dosage[a] (Kg./Ha.)	Mean No. of Larvae	
		Alive	Dead[b]
Dipterex	0.5	73.3	40.2
Toxaphene	2.9	96.3	16.6
Endrin	0.4	85.0	11.4
Ethyl parathion	0.5	77.0	9.8
Methyl parathion	0.5	72.6	2.8
Thiodan	0.5	78.3	2.8
Heptachlor	1.0	87.0	1.0
Check	—	78.0	1.8

[a] 1 kg./ha. = .89 lb./acre.
[b] Mean numbers of dead larvae not connected by the same vertical line are significantly different from each other at the 5% level of probability according to Duncan's Multiple Range Test.

the soil surface. Counts of dead larvae were subjected to analysis of variance using a square root transformation. The results obtained with the high concentrations are presented in table 4. Dipterex again gave significantly better control than other treatments, and toxaphene, endrin, and ethyl parathion killed significantly more larvae than methyl parathion, Thiodan, or heptachlor.

In plots receiving one-half the concentration of the above treatments, Dipterex, with an average kill of 30 larvae, was significantly better than the rest of the treatments, which all gave kills of less than 10 larvae.

Dipterex was the one insecticide that showed promise for the control of the salt-marsh caterpillar at the concentrations used in these two tests. Its kill was probably due for the most part to direct-contact action on the larvae rather than a residual deposit on the plants. Larvae that migrated to the plants after treatment appeared to be unaffected. Perhaps higher concentrations of Dipterex than those used might give some residual toxicity. The low mortalities obtained with ethyl parathion, methyl parathion, toxaphene, and endrin may be due to resistance to these materials within the insect population. These insecticides have been used for some years on cotton in the area and populations of *E. acrea* were observed increasing during the past three seasons in spite of the continued use of these materials.

CONCLUSIONS.—From the results obtained to date in this study of the salt-marsh caterpillar in northwest Mexico, several suggestions can be made which may help reduce damage by this species in fall-planted corn.

Since Amaranth, *A. palmeri*, and ground cherry, *P. angulata*, are the preferred native hosts both for oviposition and for larval development, more attention to the elimination of these weeds in cotton fields and in canals and drains bordering fields to be planted to corn should help reduce the population of larvae. Limiting the application of insecticides in cotton to those fields where it is absolutely necessary may enable the predatory insects to play a greater role in the reduction of caterpillar populations.

With regard to chemical control, it appears the Dipterex holds the most promise of the insecticides currently available in the area, and this material is being recommended at a slightly higher rate of application than those tested for protection of small corn from larval attack during the 1959 season.

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Control of the Corn Earworm Attacking Sweet Corn[1]

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ABSTRACT

Following a season of extensive damage to the commercial sweet corn crop, producers on Long Island became alarmed at the possibility that the corn earworm, *Heliothis zea* (Boddie), had become resistant to DDT. Experiments conducted during the next three seasons dispelled this view. Sevin® (1-naphthyl-N-methyl carbamate), used at equivalent rates, was shown to be as effective as DDT. Endrin, Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide), Guthion® (*O,O*-dimethyl *S*-(4-oxo-3H-1,2,3-benzotriazine-3-methyl) phosphorodithioate), and Shell 4402 (1,3,4,5,6,7,8-octachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanophthalan) gave promising results at the dosages used. In addition, endrin, DDT, Guthion, Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate), and ryania were found to reduce considerably ear damage caused by the European corn borer, *Pyrausta nubilalis* (Hbn.). Thiodan was partially effective.

Analyses of corn samples revealed the absence of DDT and the presence of insignificant residues of Sevin on the husked ear.

Experiments conducted concurrently with the aforementioned tests revealed that the addition of various surface-active supplements to DDT sprays apparently had little or no effect in enhancing or prolonging the effectiveness of the sprays with the possible exception of Atlas NNO which, in one test, seemed to improve control.

In the United States, the initial screening studies of Ivy (1944) and Johnson (1944) clearly indicated the effectiveness of DDT for the control of *Heliothis zea* (Boddie). Since then considerable research has been done utilizing this insecticide to control the earworm in sweet

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Regardless of the results reported herein, several of the compounds tested have not been approved for use on sweet corn and may not be used commercially.

corn. Because DDT was found to be the most consistently effective insecticide, in the Long Island area of New York State, apparently satisfactory control measures were formulated for the commercial green market sweet corn producer using DDT as the salient feature (Butler & Carruth 1949).

Until 1955, high standards of quality were generally attained, largely owing to freedom of the sweet corn ears from earworm contamination. Because of the apparent success of the earworm program, research undertakings on Long Island were suspended prior to the growing season of 1955. Unfortunately, during this season, the most serious infestation of corn earworm occurred in a decade or more. Control measures, as practiced, especially in late August, resulted in rarely more than 50% of noninfested ears. In most instances, control was considerably below this level. The producer was concerned that the earworm was becoming resistant to DDT, and the established control program seemed inadequate under conditions of high population. This prompted a resumption of the earworm control project.

The information recorded herein is in the nature of a progress report and is concerned with two phases of the total project: (1) field testing of promising insecticides, and (2) assaying the value of various adjuncts to spray formulations of DDT.

METHODS AND MATERIALS.—With the exception of the adjuvant test made during the 1956 season, all experiments were conducted at the Long Island Vegetable Research Farm at Riverhead, New York, using the variety Iochief sweet corn as the host crop. The adjuvant experiment in 1956 was carried out in cooperation with a commercial producer located at Coram, New York. The additives, listed in table 1, were tested in a duplicate-plot experiment. One of the replicates was planted to Double Duty and the other to Golden Security variety of sweet corn. Each plot consisted of four rows from 125 to 160 feet long. The sprays were applied with a commercial high-clearance sprayer at the rate of 50 gallons per acre at a pressure of 100 pounds per square inch. Three No. 65067 *TeeJet* nozzles were used per row. One was placed over the center of the row, and one on either side, at the end of drop extensions. The side nozzles were directed toward the silk region of the ear.

Applications in the insecticide test of 1956 were made with a knapsack sprayer operating at 60 p.s.i. Sprays were applied at the rate of 100 gallons per acre and were directed at the silks. Treatments were randomly distributed in each of four replicates. Each plot consisted of two rows 25.5 feet in length. Table 2 lists the compounds tested.

In 1957 and 1958, sprays were applied with a high-clearance, self-propelled sprayer. The materials applied in the insecticide tests for 1957 and 1958 are shown in table 2. The additives employed in the adjuvant tests appear in table 1. The sprayer was equipped with an adjustable boom and drop extensions. In 1957 sprays were applied at the rate of 100 gallons per acre; in 1958 at 50 gallons per acre. The sprayer was operated at a pressure of 90 p.s.i. Each treatment in the 1957 and 1958 experiments consisted of four plots in a randomized-block design. Each plot consisted of two rows from 40 to 42 feet long. Adjoining plots were separated by a buffer zone consisting of two rows of unsprayed sweet corn plants. This arrangement was designed to minimize the error caused by drift.

In 1957 the nozzle arrangement consisted of three flat spray, *TeeJet* nozzles (No. 8002) per row, one located on the horizontal boom directly over the row and one on each side of the row attached to the terminus of a drop extending downward from the boom. The lateral nozzles directed the spray toward the silk region. The following season the overhead nozzle was removed and, in its stead an additional nozzle was placed in each of the lateral positions. Thus, two pairs of nozzles were provided for each row. TX-5 *TeeJet* nozzles, with *ConeJet* tips, were substituted for the flat spray nozzles.

During the tests of 1957 and 1958, prior to silking, it was sometimes necessary to control the European corn borer, *Pyrausta nubilalis* (Hbn.), and the armyworm, *Pseudaletia unipuncta* (Haw.), using DDT or toxaphene. Since applications were made at least 2 weeks prior to the initiation of differential treatments, it was not considered likely that they would exert influence on or interfere with future earworm activity.

In 1956, and again in 1957, samples of sweet corn treated with a full schedule of DDT sprays were collected for harvest-residue determinations. Whereas only husked ears were analyzed in 1956, husks and ears were analyzed separately in 1957. In 1958, Sevin®-treated (1-naphthyl-*N*-methyl carbamate) plants were sampled for residue determinations. Two types of samples were taken: (1) unhusked ears and (2) forage.

The relative effectiveness of the various treatments was determined by examining the primary ears at harvest. One hundred ears were examined for each plot in the adjuvant test of 1956. Twenty-five ears per replicate were examined in the insecticide experiments. In 1957 and 1958, 50 ears were examined per plot. Although in,

Table 1.—The effectiveness of DDT sprays as influenced by the addition of various surface-active adjuvants.

Additive	Concentration[a]	Ears Infested				
		1956	1957		1958	
		Per Cent	Per Cent	Mean Angle	Per Cent	Mean Angle
DuPont Spreader-Sticker[b]	0.25 pt.	1.5	14.2	22.15	38.0	38.04
Colloidal X-77[c]	.25 pt.	1.5	19.1	25.94	32.0	34.44
Atlas 1045A[d]	.25 pt.	1.5	15.8	23.40		
Tergitol Anionic 7[e]	.5 pt.	2.0	13.4	21.48	38.5	38.33
Tergitol Anionic 4[e]	.5 pt.	2.5	19.9	26.47	35.4	36.53
Tergitol Anionic P-28[e]	.5 pt.	3.0	16.5	23.98	33.0	35.04
Multifilm-L[c]	.25 pt.	3.5	19.7	26.35	41.5	40.09
Plyac[f]	.5 pt.	4.0	12.8	20.39	37.4	37.69
Armour Sticker[g]	.5 lb.	11.5	17.0	24.31	33.4	35.29
Colloidal Z-1[c]	.25 lb.	17.5	20.6	26.98		
National Sticker[h]	1.0 pt.		15.1	22.89	36.2	36.96
Atlas NNO[d]	.25 pt.				25.9	30.60
DDT 7.4% plus Mineral Oil 74%[i]	1.25 lb.				30.4	33.43
None		3.5	13.7	21.76	37.5	37.75
Untreated		87.0	75.3	60.21	62.8[j]	
L.S.D. at 1% level				8.48		6.81
at 5% level				6.32		5.07

[a] Per 50 gallons in 1956 and 1958; 100 gallons in 1957.
[b] Grasselli Chemicals Dept., E. I. duPont & Co., Wilmington, Del.
[c] Colloidal Products Corp., Sausalito, Calif.
[d] Atlas Powder Co., Wilmington, Del.
[e] Union Carbide Chemicals Co., New York, N. Y.
[f] General Chemicals Div., Allied Chemical and Dye Corp., New York, N. Y.
[g] Armour and Co., Chicago, Ill.
[h] The National Sugar Refining Co., New York, N. Y.
[i] Not an additive. Dosage of actual DDT per acre is given.
[j] Not included in statistical analysis.

Table 2.—Earworm infestations in sweet corn ears subsequent to treatment with various compounds. Long Island. 1956–58.

INSECTICIDE[a,b]	ACTUAL TOXICANT (LBS./A.)	EARS INFESTED (%)	MEAN ANGLE
1956 Experiment			
DDT Em (2)	1.5	9.6	18.09
Sevin Em (2)	.5	19.2	25.98
Thiodan 25% Wp	.5	48.0	43.85
Dipterex® 50% Sp[c]	1.0	52.0	46.15
Malathion Em (5)	1.25	54.0	47.30
Endrin Em (1.6)	.2	56.0	48.46
Phosdrin Tech. (10.4)	.5	56.3	48.60
Diazinon® Em (2)[d]	.5	59.4	50.41
Union Carbide 8305 Em (4)[e]	.5	70.1	56.85
Dieldrin Em (1.5)	.375	72.3	58.23
Toxaphene Em (6)	1.5	76.4	60.92
Heptachlor Em (2)	.5	79.6	63.18
Perthane® Em (4)[f]	.5	84.1	66.50
Monsanto 11327 Em (2)[g]	.5	86.5	68.43
Chlorthion® Em (4)[h]	.5	88.8	70.47
Monsanto 10851 Em (2)[i]	.5	91.1	72.61
Monsanto 10502 Em (2)[j]	.5	92.4	74.01
Monsanto 8574 Em (2)[k]	.5	96.5	79.16
Untreated		95.9	78.33
L.S.D. at 1% level			13.68
at 5% level			10.28
1957 Experiment			
DDT Em (2)	1.5	7.7	16.11
Sevin Em (1)	.75	19.3	26.03
Sevin Em (1)	.5	30.9	33.74
Thiodan Em (2)	.5	34.0	35.64
Endrin Em (1.6)	.4	38.5	38.33
Guthion Em (1.5)	.5	48.5	44.13
Phosdrin Em (2)	.5	52.5	46.44
Malathion Em (5)	1.25	56.1	48.50
Kepone® 50% Wp[l]	1.0	65.7	54.16
Untreated		88.2	69.94
L.S.D. at 1% level			7.97
at 5% level			5.92
1958 Experiment			
Sevin Em (4)	2.0	5.9	14.08
DDT Em (2)	1.5	8.9	17.33
Sevin Em (4)	1.5	9.4	17.83
Endrin Em (1.6)	.8	19.3	26.09
Thiodan Em (2)	1.0	29.9	33.17
Shell 4402 Em (1.25)[m]	.3125	30.5	33.51
Guthion Em (1.5)	.75	35.0	36.26
Gen. Chemical 3583 Em (1.98)[n]	.5	51.0	45.59
Ryania 95% Wp	2.85	55.0	47.89
Shell 3562 Tech. (10)[o]	.5	55.7	48.26
Perthane Em (4)	1.0	57.5	49.32
Untreated		62.8	52.40
L.S.D. at 1% level			7.35
at 5% level			5.47

[a] Em, emulsifiable concentrate; Wp, wettable powder; Sp, soluble powder; Tech., technical.
[b] Numbers in parenthesis indicate pounds of active ingredient per gallon.
[c] *O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate.
[d] *O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate.
[e] *P*-chloro-2,4-dioxa-5-methyl-P-thiono-3-phosphabicyclo(4.4.0) decane.
[f] 1,1-dichloro-2,2-bis(*p*-ethylphenyl)ethane.
[g] *O,O*-dimethyl *S*-(2-propynyl) phosphorodithioate.
[h] *O*-(3-chloro-4-nitrophenyl)*O,O*-dimethyl phosphorothioate.
[i] 2-chloro-4,6-bis(diethoxyphosphinothioylthio)-*s*-triazine.
[j] 1-(dimethoxyphosphinyl) vinyl dimethyl phosphate.
[k] Tetramethyl (dithiodimethylene) diphosphonate.
[l] 1,2,3,5,6,7,8,9,10,10-decachlorotetracyclo [5.2.1.$0^{2,6}$.$0^{3,9}$.$0^{5,8}$] decan-4-one.
[m] 1,3,4,5,6,7,8-octachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanophthalan.
[n] Diethyl-1-(2,5-dichlorophenyl)-2-chlorovinyl phosphate.
[o] 3-(dimethoxyphosphinyloxy)-*N,N*-dimethylcrotonamide.

taking the data, ear infestations were categorized as to the stage of the larval instars and the extent of the injury observed, only actual percentages of infested ears are reported here.

INSECTICIDE TESTS.—*1956 Experiment.*—Applications were begun when approximately 25% of the plants were in silk. At this time, an examination of 100 silks of plants scattered throughout the plots failed to reveal earworm eggs or larvae. Three applications of insecticide were made, commencing on July 23. Sprays were applied at 4-day intervals. Ear samples were taken at random from the plots on August 10 and examined for earworm infestation and injury.

From the data in table 2, it is evident that DDT and Sevin were the most effective of the compounds tested. Under conditions of severe infestation, as in the check plots where 95% of the ears were injured, both gave very good control and were significantly superior to all the remaining treatments.

1957 Experiment.—The initial application was made on August 12 when 50% of the plants had silked. Additional sprays were applied at 2-day intervals until six applications were made. The plots were harvested on September 2, 10 days after the final application. Precipitation that occurred during and following the second spray and, abundantly, after the fifth and last applications, undoubtedly had a significant effect on the results.

The data (table 2) indicate that DDT was significantly better, at the 1% level, than all other compounds tested. Sevin, used at 0.75 pound actual per acre, was a significant improvement over the 0.5 pound rate. Unlike the findings of 1956, Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) at 0.5 pound gave control equivalent to Sevin used at a comparable rate. Endrin, at twice the dosage used in the previous season, gave only moderate control.

Secondary observations of ear injury caused by the European corn borer indicated that the greatest reductions were in the plots treated with DDT, endrin or Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate, 60% technical). Guthion® (*O,O*-dimethyl *S*-(4-oxo-3H-1,2,3-benzotriazine-3-methyl) phosphorodithioate) and Thiodan gave some reduction. The other compounds were ineffective.

1958 Experiment.—The 1958 season was favorable for rapid maturation of the sweet corn ears. The first spray was applied on August 12 when approximately 10% of the ears were in silk. Treatments were repeated at 3-day intervals, the last application being made on August 21. The plots were harvested 8 days later. Precipitation of 3.97 inches occurred between the last application and harvest and probably had some influence on control.

At a somewhat heavier dose than used in 1957, Sevin was found to give control equal to that of DDT. Both DDT and Sevin were superior to all other compounds tested. Endrin, at 0.8 pound per acre, double that of 1957, was still inadequate. Some improvement was noted with increased doses of Thiodan and Guthion, but these compounds were markedly inferior to DDT and Sevin.

Although Sevin gave good control of the corn earworm, this compound was relatively ineffective against the European corn borer attacking the ear. Endrin, DDT,

Guthion and ryania considerably reduced borer injury. Thiodan was only partially effective.

Residues.—Analysis of the husked sweet corn ears, sampled 10 days after the last application, showed an absence of DDT residues in both 1956 and 1957. However, approximately 28 p.p.m. of DDT was found on the husks taken from the ears treated in 1957. Analysis of the Sevin-treated, complete-ear sample, taken 8 days after the final application, revealed negligible quantities of the active toxicant. The residue was less than 0.12 p.p.m., which is the sensitivity of the method used in making the determinations. Slightly over 8 p.p.m. were found on the forage from which these ears were harvested.

Discussion.—The data presented in table 2, and results to be published at a later date of numerous spray-timing experiments, offer ample evidence that on Long Island the corn earworm was not resistant to DDT. It is highly probable that the difficulty of sweet corn producers during the 1955 season was caused primarily by their lack of realization of the immensity of the problem. In all known instances, spray programs were not modified to cope with the unusually high population levels.

It is noteworthy that, of all the compounds tested to date, only Sevin has equaled the performance of DDT for the control of the earworm on sweet corn.

The findings relative to DDT and Sevin residues clearly illustrate that the sweet corn ear resident within the husk is well protected from insecticide contamination. On the other hand, utmost consideration must be given to the problem of excessive residues on plant material destined for animal fodder because significant amounts of residue were found on the husks and forage.

ADJUVANT TESTS.—*1956 Experiment.*—The materials listed in table 1 were added to finished formulations comprised of 3 quarts of 25% DDT emulsifiable concentrate, containing 2 pounds of DDT per gallon, and water to make 50 gallons. Applications were made at initial silking, on September 4, and repeated on September 7, 10, 13, and 17. The ears were harvested and examined on September 22. Differences in varietal susceptibility to earworm attack were not noted. All treatments, as noted in table 1, gave significant reductions in earworm-injured ears over that found in the untreated control plots. The DDT spray containing no surface active supplement was also very effective. Thus, under conditions of this experiment, these additives did not increase the effectiveness of DDT sprays.

1957 Experiment.—The interval between applications was extended to 5 days to determine whether the supplementary surface-active agents would prolong the effectiveness of the DDT. DDT was used at the rate of 1.5 pounds per acre. The applications were made on August 11, 16, and 21. Heavy precipitation (a total of 5.42 inches) occurred between the time of the final application and harvest sampling. The results indicate that the adjuvants tested were ineffective in improving earworm control over that obtained with DDT alone. The treatment containing Colloidal Z-1 was, as in 1956, the least effective of all treatments. This may have been partially caused by the constant clogging of the sprayer nozzles when this material was applied.

1958 Experiment.—The 5-day schedule was repeated in 1958 with applications started on August 12, when approximately 10% of the plants had silked. Infestation data were obtained on August 29, one week following the last application. A commercial formulation of DDT and mineral oil[2] was included in the test to determine the relative value of the mineral oil supplement compared with other adjuvants. However, it should be noted, that 1.25 pounds of DDT were used in this treatment as against 1.5 pounds in the other treatments. At this rate, the DDT-mineral oil treatment was no better than the unsupplemented DDT spray. Under conditions of the test, none of the treatments gave adequate commercial control of the corn earworm. In part, this may have been caused by the heavy precipitation of 1.03 and 3.97 inches following the first and final applications, respectively. It is obvious that under such conditions, coupled with lengthy interspray intervals, DDT treatments may be commercially ineffectual. Atlas NNO, which was tested for the first time this season, apparently had some salutary effect on the DDT spray. This treatment gave significantly better control than the treatment which contained no supplement.

Discussion.—Fulton (1936) reported on the effective use of wetting agents in conjunction with sprays of lead arsenate for control of the corn earworm in sweet corn ears. Additional work of this nature utilizing surfactants, other than mineral oils (Barber 1939, Ditman *et al.* 1955, Eden & Ingram 1959) and others, has not been reported. The results of the tests reported here indicate, with one as yet unsubstantiated exception, that surfactants did not improve the effectiveness of DDT sprays. It is likely that there is insufficient discharge at 50 and 100 gallons per acre to soak thoroughly the silks. Consequently the deposition and retention of DDT, or its effective distribution throughout the silk bundle, is not materially assisted by the incorporation of surface-active agents.

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[2] Product of Miller Chemical and Fertilizer Corp., Baltimore, Maryland.

The Relationship of Weight of Lepidopterous Larvae to Effectiveness of Topically Applied Insecticides[1]

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Abstract

Four species of lepidopterous larvae were treated by topical application with several insecticides. LD-50 values were established on a μg. insecticide per gram of larval weight basis for a number of different weight groups for each species. The southern armyworm, *Prodenia eridania* (Cram.), gave a nearly constant LD-50 value for the different weight groups using DDT or Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate, 60% technical) as the insecticide, as did the tobacco hornworm, *Protoparce sexta* (Johan.), with endrin. The corn earworm, *Heliothis zea* (Boddie), gave no increase in the LD-50 for Phosdrin but gave more than 1,000-fold increase with DDT from larvae weighing 0.1 gm. to those weighing 0.5 gm. Of the eight insecticides tested on the tobacco budworm, *Heliothis virescens* (F.), only Phosdrin remained constant for the different size groups. Guthion® (*O,O*-dimethyl *S*-(4-oxo-3H-1,2,3-benzotriazine-3-methyl) phosphorodithioate), Dipterex® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate), and malathion gave increases of 2 to 10 times, while endrin, DDT, toxaphene and TDE give increases of from 20 to 100 times.

It is generally assumed in biological assay tests that an organism responds to toxic materials on a weight basis. In proportion to its body weight a larger animal requires a larger dose of material for extermination than a smaller animal of the same species. In most laboratory insecticide tests, efforts are made to standardize the size and age of the test insect by rearing it under controlled conditions and feeding it on a uniform food supply (Campbell & Moulton 1943). Thus, the effect of the insect's weight or age is seldom encountered in controlled studies.

During a study on the relative susceptibility of *Heliothis virescens* (F.) and *H. zea* (Boddie) to several insecticides, attempts were made to minimize the effect of larval size by applying the insecticides on a microgram per gram weight of larvae basis (Gast *et al.* 1956). That the larger larvae required a larger dose of insecticide per unit weight than the smaller larvae was immediately evident. This evidence, plus the fact that field control of large bollworms is very poor compared with the control of small worms, prompted an investigation to determine the effect of larval size on insecticidal action.

Guthrie (1954), using tobacco hornworms, *Protoparce sexta* (Johan.), found that small worms were killed by less material than were large worms; however, his results were based on micrograms of insecticide per larvae rather than micrograms per gram of larvae.

McPherson *et al.* (1956), working with *H. virescens* and *H. zea*, used individually weighed larvae and determined that the smaller larvae required less material than larger ones. The insecticide was applied at the same rate to all insects, and results were given as per cent kill rather than in LD-50 values.

Differences in the LD-50 value between small and large larvae on a weight basis for the tomato moth, *Diataroxia oleracea* L., were shown by Wray (1954). Using parathion, DDT, BHC, TEPP, and lead arsenate as stomach poisons and DDT as a contact poison, the larger larvae required up to 12 times the amount of insecticide on a weight basis as did the smaller larvae. Last instar larvae were used for all tests.

Test Insects.—Larvae of four species of lepidopterous insects were used. The tobacco budworm, *Heliothis virescens;* the corn earworm, *H. zea;* and the tobacco hornworm were field collected. The southern armyworm *Prodenia eridania* (Cram.) was laboratory reared on lima bean plants. The tobacco budworm and tobacco hornworm were collected from insecticide-free, field-grown tobacco and the corn earworm from field-grown sweet corn ears. Insects that appeared to be parasitized, diseased, or in the prepupal stage were discarded. Immediately after collection all larvae were transferred to individual 4-ounce jars, supplied with food obtained from the host plant, and held in the laboratory to await treatment.

Because of a mixed population of *Heliothis* sp. on the tobacco, all budworms were checked under a binocular microscope to verify that they were *H. virescens* and not *H. zea*. Previous work (Gast *et al.* 1956) had shown considerable differences in the susceptibility of these species to several insecticides. The presence or absence of microspines or absence of microspines on the tubercles of the first and second abdominal segments was the distinguishing characteristic.

All larvae weighing less than 1 gram were weighed to the nearest hundredth of a gram while those weighing more than 1 gram were weighed to the nearest tenth. Insects of less than 1 gram were then separated in groups to the nearest tenth of a gram and those over 1 gram into groups to the nearest gram. Each group was then randomly divided into four or five subgroups depending upon the number of rates of insecticides to be tested on the size group. No attempt was made to separate the size groups into instars as there was considerable overlapping between larval weight and larval instars. Generally there was a greater weight range within the last instar than between several preceding ones.

Insecticides and Methods.—Insecticides used in the study were DDT, Dipterex® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate), endrin, Guthion® (*O,O*-dimethyl-S-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorodithioate), malathion, Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate, 60% technical), TDE, and toxaphene. Technical grade insecticides were dissolved in methyl-ethyl ketone and applied to the

[1] Contribution from the Entomology Department, North Carolina Agricultural Experiment Station, Raleigh, North Carolina. Published with the approval of the Director of Research as Paper No. 1044 of the Journal Series. Accepted for publication May 15, 1959.

Table 1.—LD-50 values for eight insecticides topically applied on the tobacco budworm larvae.

Larva Weight in Grams	LD-50 in Micrograms of Insecticide per Gram of Larvae[a]							
	DDT	Dipterex	Endrin	Guthion	Malathion	Phosdrin	TDE	Toxaphene
0.05–0.09			10± 6	7± 3				
0.10–0.14	187± 150			7± 5	36±2.5			
0.15–0.19	315± 145		13± 9	16± 6	25± 18		2,060±1,506	
0.20–0.24	734± 201		29± 18	26± 9	33± 21		3,169±2,013	
0.25–0.29	4,294±1,315	53±41	71± 29	32±12	149± 71	3.9±1.2	7,597±2,591	7,590± 421
0.30–0.34	4,551± 927	66±28	169± 72	85±22	202±113	4.8±1.3	17,042±3,162	8,219± 6,025
0.35–0.39	7,020±3,050	63±48	327±105	86±19	140± 51	6.7±2.2	39,501±6,190	15,730±10,153
0.40–0.44	14,811±5,010	85±51	398±142	75±31	201±143	8.5±2.5	>40,000	>40,000
Regression equation[b]	$\hat{y}$=1.471 +.6553x	$\hat{y}$=1.402 +.1190x	$\hat{y}$=.412 +.5273x	$\hat{y}$=.556 +.3552x	$\hat{y}$=1.355 +.3231x	$\hat{y}$=.055 +.232x	$\hat{y}$=2.293 +.5759x	$\hat{y}$=2.435 +.4895x

[a] Fiducial limits at 5%.
[b] $\hat{y}$ = Log of LD-50.
x = average larval weight in tenths of a gram.

dorsal thoracic segments of the larvae by means of a hypodermic needle with the syringe plunger driven by a calibrated micrometer screw. With the exceptions below, concentrations of the insecticides in the methyl-ethyl ketone were such that each insect received micrometer units equal to its weight in hundreths of a gram. Thus, a larva weighing 0.75 gram would receive 75 units and a larva weighing 0.20 gram would receive 20 units. Four and one-half micrometer units were equivalent to 1 microliter. Dosages were changed by changing the concentration of the insecticide solution. By this means all larvae received solvent and insecticide in proportion to their body weight. Because it was impossible to dissolve sufficient DDT, TDE, or toxaphene in the solvent to obtain the highest desired concentration, the next lower concentration was applied at twice the volume.

Four or more concentrations were tested on each size group of larvae using 15 to 30 insects for each dosage. Treated larvae were supplied with fresh food daily and held 48 hours at 90° F. and 75% relative humidity in individudual glass containers. An insect was considered dead when it failed to move after being prodded with a needle. LD-50 values for each insecticide for each size group were determined by a probit analysis with fiducial limits at the 5% level (Finney 1952).

Regression equations were computed for each insecticide on each species of insect using the average value for each larval weight group and the log of the LD-50 value.

RESULTS AND DISCUSSION.—The test results are shown in tables 1 through 4. As the larval size increased, Phosdrin showed little increase in the LD-50 values for any of the species tested. DDT showed the greatest difference, requiring, on a microgram per gram basis, more than 1,000 times the amount on large corn earworms as on small ones. The total dose on a per larva basis would thus be more than 5,000 times more for worms over 0.5 gram as compared with worms under 0.1 gram. It was impossible to place sufficient DDT on corn earworm larvae weighing more than 0.5 gram to obtain an LD-50 value.

Little difference on a microgram per gram basis was found between hornworms from 2 grams to 7.9 grams; however, the total amount of endrin needed to kill a 7.9-gram larva was about four times that needed to kill a 2-gram larva. The sudden increase in the LD-50 values for hornworms weighing more than 8 grams can be explained partially by the fact that these larvae were approaching the prepupal stage and changes in the integument had already started that might render the integument less permeable to the insecticide.

Table 2.—LD-50 values for DDT and Phosdrin topically applied on the corn earworm larvae.

Larval Weight in Grams	LD-50 in Micrograms of Insecticide per Gram of Larvae[a]	
	DDT	Phosdrin
0.05–0.09	40± 6	3.0±0.9
0.10–0.19	70± 33	2.6±0.6
0.20–0.29	71± 34	2.4±0.5
0.30–0.39	589± 209	2.6±1.2
0.40–0.49	4,170±1,720	2.8±0.8
0.50–0.59	>40,000	3.3±1.3
0.60–0.69	>40,000	3.8±1.0
0.70–0.79	>40,000	
Regression equation[b]	$\hat{y}$=.795+.6400x	$\hat{y}$=.383+.0223x

[a] Fiducial limits at 5%.
[b] $\hat{y}$=log of LD-50.
x=average weight of larvae in tenths of a gram.

Table 3.—LD-50 values for DDT and Phosdrin topically applied on the southern armyworm.

Larvae Weight in Grams	LD-50 in Micrograms of Insecticide per Gram of Larvae[a]	
	DDT	Phosdrin
0.05–0.09	42± 7	3.9±1.0
0.10–0.19	45±10	3.1±0.8
0.20–0.29	45± 5	4.8±0.6
0.30–0.39	66±12	7.5±1.2
0.40–0.49	96±14	7.1±0.9
0.50–0.59	98± 8	5.0±1.8
0.60–0.69	115±21	5.2±1.1
0.70–0.79	86±10	
Regression equation[b]	$\hat{y}$=1.577+.0658x	$\hat{y}$=.577+.0353x

[a] = Fiducial limits at 5%.
$\hat{y}$ = log of LD-50.
x = average larval weight in tenths of a gram.

Table 4.—LD-50 values for endrin topically applied on the tobacco hornworm.

LARVAE WEIGHT IN GRAMS	LD-50 IN MICROGRAMS OF ENDRIN PER GRAM OF LARVAE[a]
0.5–0.9	0.4±0.2
1.0–1.9	0.8±0.5
2.0–2.9	1.4±1.0
3.0–3.9	1.0±0.3
4.0–4.9	1.8±0.6
5.0–5.9	1.7±0.5
6.0–6.9	1.9±0.6
7.0–7.9	2.5±1.4
8.0–8.9	4.6±1.9
Regression equation[b]	$\hat{y} = -.3070 + .104x$

[a] Fiducial limits at 5%.
[b] x = average larval weight in grams.
y = Log of LD-50.

Reasons for the difference in dosages on a microgram per gram basis cannot be explained by a simple surface to volume ratio. A larva is essentially a cylindrical body. Since the surface of a cylinder is proportional to the radius while the volume is proportional to the square of the radius, a small larva has greater surface per unit body weight than a large larvae. If the loss of effectiveness of DDT were due to a simple surface to volume phenomenon, it should be true for all species of insect larvae. However, this is not the case, as shown by the difference between the corn earworm and the southern armyworm, Whether the age or weight of the larva influences its susceptibility toward a given insecticide is difficult to ascertain, since these two factors parallel each other rather closely within a given species. The criterion used in these tests was weight because the majority of the larvae were field-collected and their exact age thus unknown. Neither age nor weight of the larva ultimately determines its susceptibility to an insecticide. Thus, physical factors, such as thickness of the integument, which may prevent penetration of the insecticide, or physiological factors such as enzymatic breakdown of the insecticide, or insecticide storage in fat bodies are probably responsible for the changes in susceptibility of the larger larvae over the smaller larvae.

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Control of Root Weevils in Strawberry Plantings

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ABSTRACT

Lindane, aldrin, heptachlor, and chlordane were tested for the control of three species of *Brachyrhinus* strawberry root weevil. Each insecticide was mixed with soil low in organic matter before setting the plants. Each of the first three was applied at the rates of 2½, 5, and 10 pounds actual per acre; the chlordane at 5, 10 and 20 pounds. After 5 years, the lindane was not giving control, the chlordane was beginning to fail, and the aldrin and heptachlor were still effective.

Root weevils have been known to destroy entire plantings of strawberries in one season (Melander & Spuler 1926). The control of root weevils, therefore, is an important part of the program for the successful production of this popular fruit. Three species are primarily responsible for the damage these insects do to the strawberry in Washington. They are the strawberry root weevil, *Brachyrhinus ovatus* (Linn.), the rough strawberry weevil, *B. rugostriatus* (Goeze), and the black vine weevil, *B. sulcatus* (Fabr.). Several other species of root weevils may injure strawberry plants in western Washington (Wilcox *et al.* 1934), but they are not numerous and, with certain exceptions, can be controlled in the same manner as the more common species.

LIFE HISTORY.—Since some investigators will not be familiar with the habits of these insects, a brief résumé of their seasonal histories follows. The life histories of the *Brachyrhinus* root weevils are similar. They usually pass the winter as white to pinkish-white more-or-less-legless grubs in the soil among the roots of the strawberry plants. However, the emergence of the adults varies considerably with the species, those of *B. ovatus* (Linn.) emerging first, followed in turn by *B. sulcatus* (Fabr.) and *B. rugostriatus* (Goeze). In the milder sections of the State, adult weevils appear throughout the summer and early fall and often overwinter in debris around the plant bases. These adults do some feeding in the fall and often lay some eggs. Overwintering adults appear again in the spring and feed on the foliage of various plants late in March or early in April. Eggs are often laid by these overwintered weevils, and failure to consider this possibility may result in poor control.

The larvae or grubs start changing to adults late in May or in June. This development can be correlated with the development of the strawberry plants. Many growers begin control measures as soon as the Marshall strawberries start to develop a red color. Adults begin laying

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eggs about 2 weeks after they emerge and continue until late August. The eggs are dropped indiscriminately in the soil about the strawberry plants, and the small grubs hatching from these eggs enter the soil to feed on the plant roots.

INJURY.—Plants that are heavily infested with root weevils become stunted and are either destroyed or the yield is reduced to a point where planting is unprofitable. In the beginning, the small grubs feed entirely on rootlets, but as they grow they feed on the larger roots and also on the crowns in heavily infested fields. Adult weevils feed on leaves and fruits, but their damage is negligible compared with that of the larvae or grubs.

CONTROL.—Until the advent of the newer insecticides control of root weevils in strawberry plantings in Washington depended on the timely application of poisoned baits to kill the adults before they could lay eggs (Melander & Spuler 1926, Downes 1927). Many growers found this method unsatisfactory and costly. Often the bait was dissipated by rain soon after application or the timing was faulty. It soon became apparent that some of the new insecticides might give better and more certain control of these pests. Work in Connecticut (Schread 1950) showed the strawberry root weevil could be controlled in nursery plantings by incorporating certain insecticides in the soil. Soon after this, it was shown that incorporating suitable insecticides in the soil in which strawberries were to be grown held definite promise as a practical and dependable means of preventing root weevil injury (Eide 1952, Pritchard 1952).

Accordingly, early in 1953 plans were developed to test the effectiveness of certain insecticides for control of root weevils when these materials were incorporated in the soil shortly before placing the transplants.

MATERIALS AND METHODS.—Aldrin, heptachlor, lindane, and chlordane, all formulated as wettable spray powders, were chosen for testing. The strawberry plants used were certain unnamed seedlings that would produce a minimum of runners, thus facilitating restriction of the plants to hills.[2]

Land was prepared for planting where irrigation water was available. As the initial step a cover crop was plowed under. Soil samples were collected from the plots and the analysis showed: Color, brown; texture, sandy clay loam; pH, 5.5; available calcium, medium; available potassium, high; available phosphorus, high; magnesium, medium; percentage of organic matter, 1.9.[3]

The plots were designed to provide 5 replications of 10 plants each for each treatment. Since root weevil populations are seldom distributed evenly over a given area or field, three sets of check plots were provided in place of the usual one—15 replications in place of 5.

Each insecticide was tested at three concentrations: The aldrin, heptachlor, and lindane were each applied at the rates of $2\frac{1}{2}$, 5, and 10 pounds actual per acre; the chlordane at 5, 10, and 20 pounds actual per acre.

The insecticides were diluted with talc in preparation for application. A garden type fertilizer spreader was used to distribute the insecticides over the plots. The quantity of material needed for each treatment was calculated, then the ability of the spreader to place that amount was checked by using the diluent alone in a test run. Each application was rotary cultivated into the soil and the transplants were placed soon afterward.

Both treated areas and the untreated checks were in bands 5 feet wide. These were separated by an untreated band 6 feet wide. The treated bands were kept free of weeds by hoeing and hand weeding, the untreated bands by longitudinal cultivation. No cross cultivation was permitted.

During the spring and summer of 1953, the following adult weevils were collected and distributed evenly over the plantings:

April 23:	90 *Panscopus torpidus* (Lec.).[4]
June 18:	169 *B. ovatus*, and 34 *B. sulcatus*.
July 1:	56 *B. ovatus*, and 81 *B. sulcatus*.
July 16:	14 *B. ovatus*, 1 *B. sulcatus*, and 22 *B. rugostriatus*.
July 21:	10 *B. ovatus*, 64 *B. sulcatus*, and 13 *B. rugostriatus*.
July 31:	66 *B. ovatus*, and 28 *B. rugostriatus*.
August 20:	11 *B. ovatus*, 39 *B. sulcatus*, and 26 *B. rugostriatus*.
August 21:	6 *B. ovatus*, 44 *B. sulcatus*, and 53 *B. rugostriatus*.
August 24:	12 *B. ovatus*, 66 *B. sulcatus*, and 129 *B. rugostriatus*.

The total is 90 *P. torpidus*, 344 *B. ovatus*, 329 *B. sulcatus*, and 271 *B. rugostriatus*. Adults of *P. torpidus* appeared in numbers in April when they were collected around recently set raspberry plants in the immediate vicinity. Adults of *B. ovatus* were collected in greatest numbers on June 18 and continued to be available throughout July and August. *B. sulcatus* adults were also collected in numbers on June 18 and continued to appear throughout the summer. In fact, numerous adults of this weevil have been found throughout the fall. They usually emerge in considerable numbers in September and many overwinter. Adults of *B. rugostriatus* did not appear until July 16, and were collected throughout the summer, appearing in greatest numbers toward the end of August. Records such as those given above provide one of the reasons why the use of poisoned baits to control root weevils in strawberry plantings has been unsatisfactory and hazardous. These collections showed that adult weevils of one or more species are present in the strawberry fields of western Washington throughout the growing season. Since these adults are all females capable of laying numerous eggs, they are a continuous threat to crop welfare.

RESULTS.—By 1958 many grubs were present among the roots of the plants in the untreated checks and many plants were all but cut off from the soil. Moreover, moles had begun working in the untreated check plots and were particularly active in one. Several of the insectivorous animals were trapped, but it is reasonable to assume they may have eaten many grubs. It was also noted that the moles had avoided the treated plots. This was not caused entirely by the absence of grubs, for they were numerous in the lindane-treated plots.

Early in the year, each plant was lifted and the soil still held by the roots, together with the other soil in which the plant had been growing, was carefully screened for grubs. All grubs found were preserved in alcohol and counted. In general, the three species of *Brachyrhinus* with which the plots had been "seeded" were present in about the same ratio as in the initial "seeding." The results are shown graphically in figure 1 and are in line with those obtained by workers (Eide 1955, Andison 1956) in

[2] The strawberry plants were furnished by Dr. C. D. Schwartze, Horticulturist, Washington Agricultural Experiment Stations.

[3] Soil analyses were by Dr. C. B. Harston, Extension Soils Specialist, Washington State University.

[4] This weevil was found feeding on the leaves of raspberries. It apparently failed to become established on the strawberries.

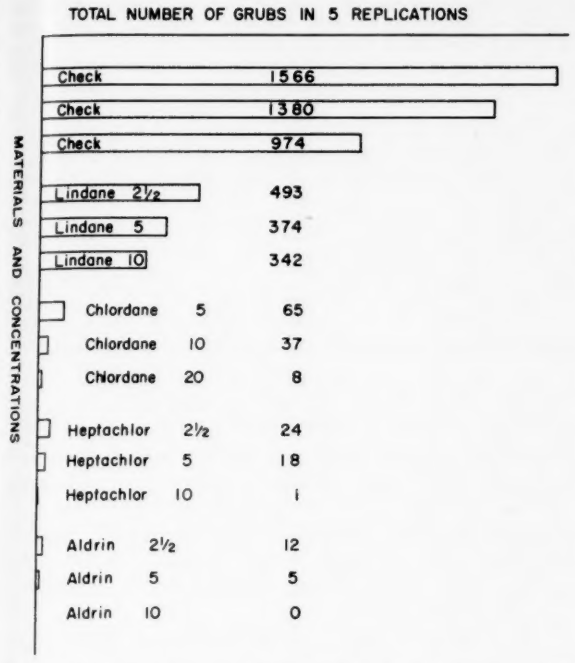

FIG. 1.—Root weevil control in strawberries with soil treatments 5 years after application, Puyallup, Washington, 1953–58.

other areas where climatic conditions are similar to those prevailing in the Puyallup Valley. While their tests were of shorter duration, the results support those considered here.

It should be noted that the organic matter in the soil was low, 1.9%. This fact may help explain the "longevity" of the insecticides in the soil. Recent studies in Wisconsin (Edwards *et al.* 1957) show that aldrin and lindane are inactivated faster in soils rich in organic matter, such as muck and loam, than they are in sand and sandy loam soils low in organic matter. Bioassay showed that the insecticides became adsorbed and lost their toxicities. These losses were correlated with the percentages of organic matter in the soil. The exact nature of the interaction between the organic matter and the insecticide was not determined, but it appeared to be an adsorption effect, since the extracted insecticide still retained insecticidal activity. Lindane and aldrin are known to produce degradation products so the organic matter in the soil probably adsorbed the degradation materials just as efficiently as it did the insecticide itself. In general, the degradation products of lindane are known to be relatively nontoxic while those of aldrin are usually more toxic than aldrin itself. When samples of aldrin-treated soils were submitted to dieldrin analyses, it was found that a considerable portion of the aldrin had been converted to dieldrin.

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Insecticide Residues on Sweet Potatoes[1]

JAMES E. ROBERTS[2], MINTER DUPREE[2], and L. H. DAWSEY[3]

ABSTRACT

Chlorinated hydrocarbon insecticides were applied to the soil and to sweet potato foliage each year from 1955 through 1958. Insecticide residue determinations were made by the methods cited from washed and unwashed samples. Data from plots receiving recommended rates of insecticides showed that residue values from unwashed potatoes were usually within tolerance limitations. Residues found on washed potatoes were much below tolerance levels.

The evaluation of insecticide control of sweet potato insects has been in progress at the Georgia Experiment Station for the past several years. Passage of the Miller Amendment made it evident that any insecticide resulting in residues in excess of approved tolerances could not be used on crops entering into interstate commerce. Hence, insecticide residue investigations on sweet potatoes were initiated in 1954 (Dupree *et al.* 1955) in conjunction with the insect-control research program. In tests in which heptachlor, aldrin, dieldrin, toxaphene, and DDT were applied to the soil in insecticide-fertilizer mixtures, only DDT gave a residue on harvested sweet potatoes.

Studies were continued to obtain further information on insecticide residues on sweet potatoes. This paper is a

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Table 1.—Insecticide residues from unwashed sweet potatoes grown in soil treated with granulated insecticides applied in the drill with fertilizer before planting, Experiment, Georgia, 1955.

Insecticide	Concentration (%)	Pounds Active Ingredient	Residue (p.p.m.)
Heptachlor	2.5	2	0.11
Aldrin	2.0	2	.06
Dieldrin	5.0	2	.06
Toxaphene	20.0	10	.11
DDT	5.0	5	.01

report of 4 years' work comprising the seasons of 1955 through 1958.

Methods and Results.—Most of the residue data reported in this paper are from samples analyzed at the Tifton, Georgia, Laboratory by the Phillips (1954) total chlorine method. However, the higher aldrin determinations in 1958 were checked and corrected by means of the Shell (Anonymous 1955) phenyl azide method. The 1957 and 1958 heptachlor data are from samples analyzed spectrophotometrically (Ordas *et al.* 1956) by the Velsicol Chemical Corporation. Residue data from each treatment are based on an average of four samples harvested from replicated plots. Determinations reported for the first 3 years are from unwashed potatoes. The 1958 determinations were made from washed potatoes.

Data for 1955 are from tests in which hand-mixed materials were applied in the row, at the rate of 500 pounds of 4-8-8 fertilizer plus the desired pounds of actual toxicant per acre. These materials were applied and plants of the Leeland Bunch variety of sweet potatoes were set April 22. Potatoes were harvested September 21. Total rainfall from planting until harvest time was 28.19 inches. The residue data from this test are presented in table 1.

In 1956, sweet potato residue data were obtained from aldrin and heptachlor applications used as (1) soil treatments, (2) soil treatments plus dust treatments, and (3) dust treatments only. The soil treatments were a hand-mixed insecticide-fertilizer applied in the row at the rate of 2 pounds of actual toxicant plus 600 pounds of 4-12-12 fertilizer per acre. This material was applied and plants of the Goldrush variety of sweet potatoes were set

Table 2.—Insecticide residues from unwashed sweet potatoes receiving soil treatments, soil treatments plus dust treatments and dust treatments only, Experiment, Georgia, 1956.

Treatment	Pounds Actual Insecticide per Acre Applied			Residue (p.p.m.)
	to Soil		to Foliage	
Soil only				
Aldrin	2			0.13
Heptachlor	2			.00
Soil+Foliage				
Aldrin	2	+	4	.06
Heptachlor	2	+	4	.53
Foliage only				
Aldrin			4	.03
Heptachlor			4	.00

Table 3.—Insecticide residues from unwashed sweet potatoes receiving soil treatments and soil plus foliage treatments. Experiment, Georgia, 1957.

Treatment	Pounds Actual Insecticide per Acre Applied			Residue (p.p.m.)
	to Soil		to Foliage	
Soil only				
Aldrin	2			0.04
Heptachlor	2			.00
Soil+foliage				
Aldrin	2	+	5	.00
Heptachlor	2	+	5	.10

May 31. Dusts at the rate of 0.5 pound per acre for each application were applied as foliage treatments with a small hand duster. Dusts were applied at 7-day intervals for a period of 8 weeks beginning July 25 and ending September 17. Potatoes were harvested October 16. Total rainfall from planting until harvest time was 18.72 inches. Rainfall from the first dust treatment until harvest totaled 13.99 inches. Residue data from this test are reported in table 2.

The 1957 sweet potato residue data were obtained from aldrin and heptachlor applications used as soil treatments only and as soil treatments plus dust treatments. Soil treatments were a commercial insecticide-fertilizer mixture (0.33 pounds of toxicant per 100 pounds of 4-12-12 fertilizer) applied in the row at the rate of 600 pounds per acre. This material was applied and plants of the Georgia Red variety of sweet potatoes were set on April 29. Beginning June 25, applications of dusts were applied weekly to plots receiving foliage treatments. Dusts were applied with a small hand duster at the rate of 0.5 pound per acre at each application. Ten applications were made. Potatoes were harvested October 5. Weather records show a total of 20.31 inches of rainfall between planting and harvest and 11.1 inches between the first dust treatment and harvest. Residue data from this test are presented in table 3.

In 1958, soil and foliage treatments were not combined as previously. Materials and methods for the insecticide-fertilizer mixtures and also the dust applications were identical with those used in 1957. Two pounds of actual toxicant per acre were applied in the row in soil treatments. Soil treatments were applied and the plants of Georgia Red variety of sweet potatoes were transplanted on May 15. Eight weekly dustings were applied to the foliage treatments beginning on July 15 and ending on August 27. Potatoes were harvested October 8. Rainfall from date of transplanting to date of harvest totaled 21.91 inches and rainfall between the date of first dust treatment and date of harvest was 8.16 inches. Residue data from this test are presented in table 4.

Discussion.—Aldrin and heptachlor are currently recommended in Georgia at the rate of 2 pounds of actual toxicant per acre for control of soil insects attacking sweet potatoes. The approved Food and Drug Administration tolerance on raw sweet potatoes is 0.1 p.p.m. for aldrin and heptachlor. According to personal communications with local marketing specialists, most of the sweet potatoes grown in Georgia are washed before shipping.

Table 4.—Insecticide residues from washed sweet potatoes receiving soil treatments and foliage treatments. Experiment, Georgia, 1958.

Treatment	Pounds Actual Insecticide per Acre Applied		Residue (p.p.m.)
	to Soil	to Foliage	
Soil only			
Aldrin	2		<0.01[1]
Heptachlor	2		<0.03
Foliage only			
Aldrin		4	<0.01
Heptachlor		4	<0.01

[1] Limit of sensitivity of the method.

Data from 1955 and 1956 (tables 1 and 2) show residues slightly in excess of approved tolerances. Since these analyses were made by the total chlorine method, they cannot be compared directly with the later data based on the colorimetric method. The values resulting from total chlorine determination can probably be considered as maximum amounts of the heptachlor or aldrin and/or their epoxides and decomposition products. The 0.53 p.p.m. heptachlor recorded in table 2 is from experimental applications which are not in agreement with dosages currently recommended to the farmer. It may be noted also that the data presented in tables 1, 2, and 3 are from unwashed potatoes. Residues on washed potatoes in 1958 were much below tolerance levels (table 4).

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Alkylphosphonic Acid Esters as Insecticides[1,2]

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ABSTRACT

A large number of alkyl *p*-nitrophenyl alkylphosphonates and their sulfur analogs have been prepared and examined for insecticidal activity. A large majority of these compounds were extremely effective against the house fly, *Musca domestica* L., in spite of wide variations in both alkyl groups. The insecticidal activity of these compounds appears to be largely a function of the reactivity of the molecule.

Despite the very high toxicity of certain alkyl phosphonates such as isopropyl methylphosphonofluoridate (sarin), 3,3-dimethyl-2-butyl methylphosphonofluoridate (soman), and cholyl methylphosphonofluoridate to mammals (Fredriksson 1957), little attention has been given to the utilization of such phosphonates as insecticides. Of the 30 or more organophosphorus insecticides now in commercial usage only two, *O*-ethyl *O*-*p*-nitrophenyl phenylphosphonothioate (EPN) and *O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate (Dipterex® or Dylox®), are esters of phosphonic acids; the others are all derivatives of phosphoric acids.

Schrader as early as 1947 described several *O*-ethyl *O*-substituted phenyl methylphosphonates and gave fragmentary information as to their insecticidal activity. The writers have recently examined a series of *O*-ethyl *O*-*p*-nitrophenyl alkylphosphonates where the alkyl group was systematically varied from methyl through hexyl with straight and branched chains (Fukuto & Metcalf 1959). It was found that a number of these compounds were extremely toxic to the house fly, *Musca domestica* L. Since this work began, several Russian papers have described the insecticidal potency of some of the compounds reported here (Paikin *et al.* 1957, Razumov *et al.* 1957. Compounds II, XX, XXI, XXII and XXVI). This paper reports the results of a systematic examination of the insecticidal properties of the alkylphosphonate esters together with considerations of the relationship of structure to activity and a general appreciation of the possibilities in this field.

MATERIALS AND METHODS.—Some of the compounds reported in this paper were described by Fukuto & Metcalf 1959. The preparation of others is reported here.

Dialkyl alkylphosphonate.—Diethyl ethylphosphonate was obtained from the Virginia-Carolina Chemical Corporation, Richmond, Virginia, and used without any purification. Di-*n*-propyl propylphosphonate, di-*iso*propyl ethylphosphonate, diethyl allylphosphonate and diethyl 2-ethoxyethylphosphonate were prepared by reacting the appropriate alkyl halides and trialkyl phosphates in the Michaelis-Arbuzov reaction (Kosolapoff 1950).

Alkyl alkylphosphonochloridate.—The above-mentioned dialkyl alkylphosphonates were converted to the corresponding alkyl alkylphosphonochloridate by treatment with phosphorus pentachloride according to the procedure of Kabachnik & Rossiiskaya (1946). The phosphonochloridates were obtained as slightly impure products. Formation of varying amounts of the phosphono-

[1] Paper No. 1160, University of California Citrus Experiment Station, Riverside. Accepted for publication May 25, 1959.

[2] Supported in part by a research grant from the Monsanto Chemical Company, St. Louis, Missouri.

dichloridate was unavoidable and this material could not be separated by distillation; therefore, the products were not characterized, but carried on through to the final phosphonate esters. Boiling points of the crude uncharacterized alkyl alkylphosphonochloridates are given in table 1.

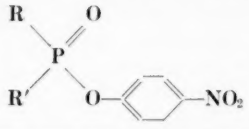
S-Alkyl alkylphosphonochloridothiolate.—All *S*-alkyl alkylphosphonochloridothiolates were prepared by the reaction between the appropriate alkyl mercaptan, alkylphosphonodichloridate (prepared according to Kabachnik & Rossiiskaya 1946), and triethylamine in ether. The following procedure for the synthesis of *S*-ethyl *n*-propylphosphonochloridothiolate is typical. To a stirred mixture of 18.5 gm. *n*-propylphosphonic dichloride and 50 ml. ether was added an ether solution of 7.2 gm. ethyl mercaptan and 16.4 gm. triethylamine. The flask was cooled in ice during the addition. The mixture was stirred for 3 hours at room temperature, filtered, ether removed, and the product was distilled, b.p. 94–98 (0.5 mm.). The yield was 5.2 gm.

As in the case of the alkyl alkylphosphonochloridates, these intermediates were not characterized, but were converted directly to the final esters. Table 1, part b lists their boiling points.

Ethyl ethylphosphonochloridothionate and ethyl *n*-butylphosphonochloridothionate were prepared according to Razumov *et al.* (1952).

Phosphonate, phosphonothiolate, and phosphonothionate

Table 1.—Physical constants of new phosphonate esters.



R	R′	B.P. of Intermediate Alkyl Alkylphosphonochloridate, °C.	B.P. °C.	n_D^{30}	Formula	Analyses (%) Calcd.	Found
A. Alkyl *p*-nitrophenyl alkylphosphonates and phosphonothioates							
n-C_3H_7	O-*n*-C_3H_7	90–92 (4 mm.)	144–6 (0.2 mm.)	1.5108	$C_{12}H_{18}O_5NP$	C 50.16 H 6.32	C 49.83 H 6.76
C_2H_5	O-*i*-C_3H_7	73–78 (10 mm.)	135 (0.05 mm.)	1.5117	$C_{11}H_{16}NO_5P$	C 48.36 H 5.90	C 48.57 H 6.42
Allyl	OC_2H_5	76–82 (6 mm.)	138–40 (0.1 mm.)	1.5262	$C_{11}H_{14}NO_5P$	C 48.75 H 5.20	C 48.63 H 5.53
$ClCH_2$	OC_2H_5	94–96 (7 mm.)	146–50 (0.07 mm.)	1.5356	$C_9H_{11}ClNO_5P$	C 38.66 H 3.97	C 39.39 H 4.60
$C_2H_5OCH_2CH_2$	OC_2H_5	98–102 (3 mm.)	150 (0.05 mm.)	1.5116	$C_{12}H_{18}NO_6P$	C 47.51 H 5.97	C 47.75 H 6.78
C_2H_5	SC_2H_5	86–100 (2 mm.)	145 (0.1 mm.)	1.5623	$C_{10}H_{14}NO_4PS$	C 43.62[a] H 5.13	
C_2H_5	S-*n*-C_3H_7	96–100 (1 mm.)	145–50 (0.05 mm.)	1.5540	$C_{11}H_{16}NO_4PS$	C 45.66[a] H 5.58	
C_2H_5	S-*i*-C_3H_7	76 (0.6 mm.)	140 (0.05 mm.)	1.5524	$C_{11}H_{16}NO_4PS$	C 45.66[a] H 5.58	
n-C_3H_7	SC_2H_5	94–98 (0.5 mm.)	150–5 (0.2 mm.)	1.5595	$C_{11}H_{16}NO_4PS$	C 45.66 H 5.58	C 45.65 H 6.18
B. Alkyl *p*-nitrophenyl alkylphosphonothionate							
C_2H_5	OC_2H_5	76–80 (10 mm.)	m.p. 38–40	—	$C_{10}H_{14}NO_4PS$	C 43.63 H 5.13	C 43.24 H 5.68
n-C_4H_9	OC_2H_5	75–81 (1.2 mm.)	140 (1.0 mm.)	1.5400	$C_{12}H_{18}NO_4PS$	C 47.52 H 5.97	C 47.72 H 6.29

[a] Proper elemental analyses for these compounds could not be obtained due to incomplete combustion. However, they all liberated the theoretical quantity of *p*-nitrophenol in phosphate buffer.

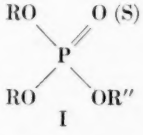
esters of p-*nitrophenol.*—The compounds listed in section a of table 1 were prepared according to the procedure described by Fukuto & Metcalf (1959). The two phosphonothionate esters in section b were prepared in the same manner except methyl ethyl ketone was used as the solvent.

Ethyl S-p-*nitrophenyl ethylphosphonothiolate* was prepared according to Fukuto & Metcalf (1959) from ethyl ethylphosphonochloridate and *p*-nitrothiophenol (Price & Stacey 1946). The compound distilled at 145° C. (0.2 mm. in falling-film molecular still), n_D^{30} 1.5721 elemental analyses: per cent calculated for $C_{10}H_{14}NO_4PS$, C = 43.62, H = 5.13; found, C = 43.20, H = 5.73.

The methods used to determine first-order rates of hydrolysis ($K_{hyd.}$) in 1/15 *M* disodium hydrogen phosphate and second-order rates of reaction (K_e) with fly-brain cholinesterase have been described by Fukuto & Metcalf (1959).

Toxicity tests to insects were made using standard w/v solutions in acetone. These were topically applied as 1 microliter droplets to adult *M. domestica* and *Periplaneta americana* (L.) (March and Metcalf 1949); as 1 ml. of solution to 100 ml. water containing 20 4th-instar *Culex quinquefasciatus* (Say) larvae; as surface residues on mature Valencia oranges by dipping these in the acetone solutions, for adult female *Panonychus citri* (McG.), and *Heliothrips haemorrhoidalis* (Bouché) (Metcalf 1949); and as surface residues to 8.5 cm. diameter cotton-leaf discs by pipetting 1 ml. of acetone solution and adding 5 fourth instar *Estigmene acrea* (Drury) larvae. Mortality was observed after 24 hours except for *Periplaneta*, when the interval was 120 hours.

DISCUSSION OF RESULTS.—*Relation of Structure to Toxicity.*—This subject is one of the most fascinating aspects of the study of the insecticidal activity of organophosphorus compounds. In the case of orthophosphate esters and their sulfur analogs, thousands of highly toxic substances are known, yet these are nearly all esters of dialkyl phosphoric acid (I) where the alkyl group R is methyl, ethyl, propyl, or isopropyl. Longer chain alkyl esters are of greatly reduced toxicity. Thus, the toxicity of insecticides of type I is dependent largely on the nature of the group R″. With the comparable series of phosphonate esters studied here (II) one of the alkoxy groups is replaced by an alkyl group (R′) resulting in a P—C bond. This group can be varied in a number of ways (table 1) without greatly reducing the toxicity. The number of highly active toxicants, therefore, in the phosphonate series is far greater than that of the orthophosphate esters. By varying R″ in addition to R′ in compounds of type II, the possible number of active toxicants becomes enormous.



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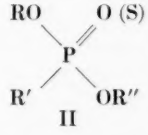
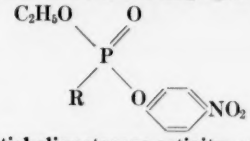


II

Of further interest is the optical activity exhibited by phosphonates or phosphonothioates when the central phosphorus atom is surrounded by four different groups, thus becoming asymmetrical. Fukuto & Metcalf (1959a) have shown that the *levo*-isomer of *O*-ethyl *S*-2-(ethylthio)ethyl ethylphosphonothiolate is 6 to 10 times as toxic to insects as the *dextro*-isomer.

Table 2.—The effect of the alkyl group R in



on toxicity, anticholinesterase activity, and rate of hydrolysis.

R=	LD_{50} *Musca*	K_e	$K_{hyd.}$
I. CH_3	1.0	7.6×10^6	2.42×10^{-3}
II. C_2H_5	1.2	1.5×10^7	1.20×10^{-3}
III. *n*-C_3H_7	2.0	1.0×10^7	4.17×10^{-4}
IV. *n*-C_4H_9	2.4	4.0×10^6	4.23×10^{-4}
V. *n*-C_5H_{11}	12.0	8.9×10^5	3.62×10^{-4}
VI. *n*-C_6H_{13}	62.0	6.9×10^5	3.56×10^{-4}
VII. *i*-C_3H_7	28.0	1.1×10^5	1.07×10^{-4}
VIII. *i*-C_4H_9	9.0	3.9×10^5	2.34×10^{-4}
IX. *i*-C_5H_{11}	12.0	4.9×10^6	2.45×10^{-4}
X. *i*-C_6H_{13}	40.0	2.0×10^6	3.62×10^{-4}
XI. *t*-C_4H_9	>5000	6.7	3.2×10^{-6}
XII. $CH_2{=}CHCH_2$	1.2	7.8×10^6	1.54×10^{-3}
XIII. $C_2H_5OC_2H_4$	12.0	3.1×10^5	2.34×10^{-3}
XIV. $ClCH_2$	28.0	2.7×10^6	3.49×10^{-2}
XV. $Cl(CH_2)_3$	8.5	5.9×10^5	7.53×10^{-3}
XVI. Cyclohexyl	>500	6.1×10^4	3.07×10^{-5}
XVII. $C_6H_5CH_2$	>500	1.9×10^5	7.05×10^{-4}
XVIII. C_6H_5	2.8	1.8×10^6	2.64×10^{-3}
XIX. OC_2H_5 (para-oxon)	1.2	2.7×10^7	6.14×10^{-5}

The insecticidal activity of alkyl *p*-nitrophenyl alkylphosphonate and phosphonothioate esters will be discussed here from the standpoint of the topical LD_{50} values to *M. domestica* resulting from systematic variations of various portions of the molecule. Thus, in compounds of type II where R″ is *p*-nitrophenyl, the effect on toxicity of variations in R and R′, and the interchange of sulfur for oxygen are examined.

Variations in the Alkyl Group R.—A previous paper concerning phosphonate esters (Fukuto & Metcalf 1959) summarized the results of varying group R in ethyl *p*-nitrophenyl alkylphosphonates on the general reactivity of the molecule measured in terms of first-order hydrolysis constants $K_{hyd.}$ in phosphate buffer, bimolecular rate constants K_e for reaction with fly-brain cholinesterase, and topical LD_{50} to *M. domestica*. This series of compounds has been extended in this paper and emphasis is placed on the correlation of toxicity with measurements of reactivity. The toxicity data for compounds with a wide variety of R groups are given in table 2. In the straight chain compounds there is a slight decrease in activity from methyl (I) to *n*-butyl (IV) although all of these compounds are extremely toxic. These compounds are equal to or better than para-oxon in activity. The toxicity drops abruptly for the *n*-amyl (V) compound and *n*-hexyl (VI) compounds. There is also a consistent drop in the anticholinesterase activity of these compounds. Of further interest is the relationship obtained when the topical LD_{50} is plotted against Taft's polar substituent constant σ^* as shown in figure 1 (Taft 1953).[3] It

[3] Values of σ^* are undetermined for the longer branched chain substituents. These have been approximated by adding the value of the single methylene group (0.015) to the nearest homologue.

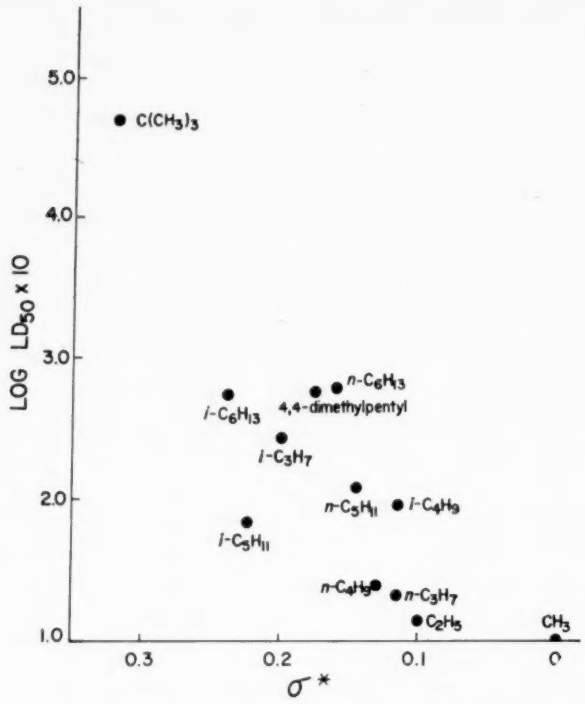


FIG. 1.—A plot of log LD_{50} to the common house fly *versus* Taft's polar substituent constant σ^*.

appears that the change in toxicity of the straight and branched chain compounds are consistent with the relative reactivities of these esters as calculated by the polar substituent constants.

The results with the branched chain compounds are somewhat more complex, but are consistent with known steric influences on the reactivity of phosphonic and carboxylic acid esters (Fukuto & Metcalf 1959). The effect of branching from *n*-propyl (III) to *iso*propyl (VII), forming a secondary carbon atom on phosphorus, decreased the toxicity 14-fold. The presence of a tertiary carbon atom on phosphorus such as in the *tert*-butyl ester (XI) produced an inert compound with essentially no insecticidal activity. It is perhaps most remarkable that a change in the alkyl group from *n*-butyl (IV) to *tert*-butyl (XI) resulted in a decrease in toxicity of over 2000-fold. The effect of branching in the 2, 3 and 4 carbon atoms, as in the isobutyl (VIII), isoamyl (IX) and isohexyl esters (X), on toxicity is less apparent. Steric inhibition is still present with the isobutyl ester since this compound is about a fourth as effective as the *n*-propyl (III) and *n*-butyl (IV) ester. The toxicity of the isoamyl and isohexyl esters is very close to their straight chain isomers and steric factors do not appear important here.

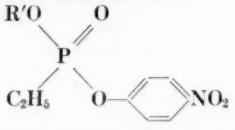
The presence of unsaturation in the group R, such as in allyl and phenyl, favors increased toxicity over the corresponding saturated groups. This is probably due to increased reactivity of the compound toward optimum values caused by electron-withdrawal by the unsaturated systems. Thus, allyl (XII) was nearly twice as effective as *n*-propyl (III), and phenyl (XVIII) was several hundred times as effective as cyclohexyl (XVI). The ω-chloroalkyl compounds were, however, considerably reduced in effectiveness, chloromethyl (XIV) about 1/28 that of methyl (I), and chloropropyl (XV) about 1/4 that of propyl (III). The decreased effectiveness of the chloropropyl compound is in line with its lower anticholinesterase activity. This relatively low activity has been explained by repulsion between the chlorine atom in the three position and the anionic site on the enzyme surface (Fukuto & Metcalf 1959). The chloromethyl compound is quite susceptible to aqueous hydrolysis; in this case beyond the point where optimum toxicity is obtained. The compound is probably largely degraded *in vivo* before it can exert its toxic action.

Variations in the Alkyl Group R'.—It is well known from studies of the tetraalkyl pyrophosphates (Toy 1948, 1950) and dialkyl *p*-nitrophenyl phosphates (Metcalf & March 1949) that toxicity of phosphoric acid esters is correlated with the nature of the alkyl group and in general decreases rapidly with increasing chain length so that the isopropyl and *n*-butyl esters are of low activity. With the alkyl *p*-nitrophenyl ethylphosphonates this effect is much less evident and toxicity is only slightly decreased when the alkyl chain increases from C_1 to C_4 and is still relatively high in the C_6 and C_8 compounds (table 3). It is apparent from the data in table 3 that variations of R^1 in the alkoxy moiety do not alter the effectiveness of the compound as much as variations of R in ethyl *p*-nitrophenyl alkylphosphonates (table 2). The effect of a wide variety of alkyl groups on the reactivity of alkyl *p*-nitrophenyl ethylphosphonates is under current investigation and will be discussed in detail in a subsequent paper.

Sulfur Analogs.—The general effect of the substitution of P═S for P═O in the phosphoric acid esters is to decrease toxicity slightly and to increase hydrolytic stability, *e.g.*, para-oxon is more effective against house flies than parathion but is much less stable to aqueous hydrolysis. However, among the three phosphonothionates examined, two showed higher toxicity than the corresponding phosphonates (table 4). Ethyl *p*-nitrophenyl ethylphosphonothionate (XXVII) and EPN (XXXII) were both slightly more toxic than their oxygen analogs. The former was the most effective member of the entire series of compounds. In comparison, the butylphosphonothionate (XXXI) was about half as effective as its oxygen analog (IV).

The toxicity of parathion, a poor anticholinesterase *in vitro*, to insects and mammals has been attributed to its

Table 3.—Effect of variations in alkyl group R' in alkyl *p*-nitrophenyl ethylphosphonates,



R=	LD_{50} *Musca*
XX. CH_3	1.2
II. C_2H_5	1.4
XXI. *i*-C_3H_7	1.5
XXII. *n*-C_4H_9	1.45
XXIII. *n*-C_6H_{13}	5.4
XXIV. *n*-C_8H_{17}	14.5
XXV. *n*-$C_{10}H_{21}$	120.0

Table 4.—Comparison of *O*- and *S*-analogs.

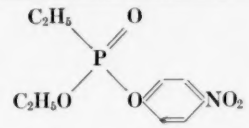
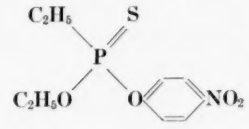
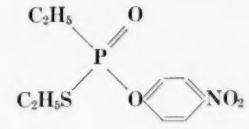
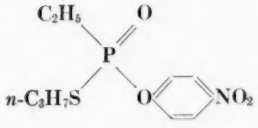
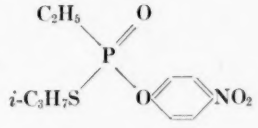
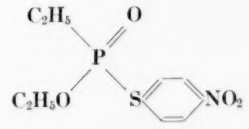
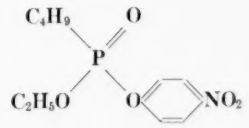
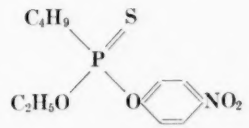
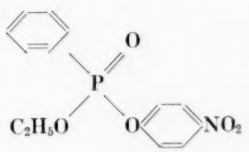
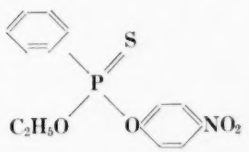
		LD_{50} *Musca*	K_e	$K_{hyd.}$ (MIN.$^{-1}$)
II.		1.4	1.5×10^{7}	1.2×10^{-3}
XXVI.		0.93	6.9×10^{2}	3.9×10^{-5}
XXVII.		8.5	2.7×10^{6}	3.82×10^{-2}
XXVIII.		9.5	6.9×10^{5}	2.76×10^{-2}
XXIX.		8.5	3.0×10^{6}	1.05×10^{-2}
XXX.		24.0	6.0×10^{6}	5.7×10^{-3}
IV.		2.4	4.0×10^{6}	4.23×10^{-4}
XXXI.		5.5	6.8×10^{2}	3.9×10^{-6}
XVIII.		2.8	1.8×10^{6}	6.2×10^{-5}
XXXII.		1.9		

Table 5.—Cross resistance of *O*-ethyl *O*-*p*-nitrophenyl ethylphosphonothionate to resistant house flies.

Compound	Topical LD (γ per Female Fly)					
	S-Strain		Chlorinated Hydrocarbon R		Phosphate R	
	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}
O-ethyl *O*-*p*-nitrophenyl ethylphosphonothionate (XXVI)	0.018	0.029	0.039	0.055	0.053	0.092
parathion	0.018	0.033	0.023	0.043	0.053	0.086
Chlorthion	0.33	0.5	0.33	0.5	6	60
malathion	0.56				1.0	40
DDT	0.033		>100		>100	

in vivo oxidation to para-oxon, a powerful anticholinesterase (Metcalf & March 1953; Gage 1953). Furthermore, the extremely low anticholinesterase activity of parathion has been attributed to increased stability of the P—O—$C_6H_4NO_2$ bond owing to the presence of the sulfur atom. Para-oxon has been shown to hydrolyze about 10 times faster in dilute sodium hydroxide than parathion (Williams 1951). Table 4 shows that ethyl *p*-nitrophenyl ethylphosphonothionate (XXVI) is hydrolyzed about 30 times slower than its oxygen analog (II) in 1/15 *M* disodium hydrogen phosphate (pH 8.6). Also, both EPN and ethyl *p*-nitrophenyl butylphosphonothionate (XXXI) were relatively stable to hydrolysis in the same buffer compared to their oxygen analogs (table 1). The low cholinesterase inactivation rates shown by the three phosphonothionates compared to their oxygen analogs (order of 10^4 slower) indicate that, like parathion and other phosphorothionates, these compounds appear much too stable to react with cholinesterase at an appreciable rate. Clearly, an activation process, presumably the conversion of the P=S to the P=O moiety, also occurs with phosphonothionates in animals during intoxication.

The *S*-alkyl phosphonothiolates, *e.g.*, *S*-ethyl (XXVII), *S*-*n*-propyl (XXVIII) and *S*-isopropyl *p*-nitrophenyl ethylphosphonothiolates (XXIX) were approximately one-sixth as toxic to house flies as their oxygen analogs. Unlike the phosphonothionates the *S*-alkyl compounds were very effective inhibitors of cholinesterase. The sixfold drop in toxicity is probably due to their relatively high susceptibility to aqueous hydrolysis. The *S*-ethyl and *S*-isopropyl compounds are 32 and 50 times less stable in phosphate buffer than their respective oxygen analogs.

Ethyl *S*-*p*-nitrophenyl ethylphosphonothiolate (XXX) was even less effective than the *S*-ethyl isomer (XXVII) against house flies in spite of comparable (slightly better) anticholinesterase activity. It is not clear why this compound is the least effective isomer since the phosphorylating part of the molecule (ethyl ethylphosphonyl moiety) is exactly the same as the oxygen analog. From its inhibition and hydrolysis rates one would predict its insecticidal activity to be very close to the phosphonate and certainly better than its *S*-ethyl isomer.

Toxicity of Alkylphosphonate Esters to Resistant Strains of House Flies.—In evaluating any new toxicant today, the effectiveness to resistant strains of test insects is of major importance. Preliminary data on this factor for *O*-ethyl *O*-*p*-nitrophenyl ethylphosphonothionate (XXVI) was obtained by measuring the topical LD_{50} and LD_{95} values for this compound to a strain highly resistant to both DDT-type and cyclodiene-type chlorinated hydrocarbons (Super Pollard), and a strain resistant to malathion and Chlorthion® (*O*-(3-chloro-4-nitrophenyl) *O*,*O*-dimethyl phosphorothioate) (Stauffer Chlorthion). The results (table 5) clearly indicate that the chlorinated hydrocarbon resistant strain (Super Pollard) is almost completely susceptible to both parathion and compound XXVI; the slight increase in LD_{50} values being the result of the increased size and vigor of the Super Pollard flies. The Stauffer Chlorthion strain, which is very highly resistant to Chlorthion and malathion especially at the LD_{95}, is only about three times as resistant to both parathion and compound XXVI. This is further demonstration of the specificity of resistance to the organophosphorus insecticides, a factor of great importance in planning for the future control of resistant species.

Toxicity of Alkylphosphonate Esters to Other Insects.—Routine screening tests were made to compare the effectiveness of *O*-ethyl *O*-*p*-nitrophenyl propylphosphonate (III) and *O*-ethyl *O*-*p*-nitrophenyl ethylphosphonothionate (XXVI) with para-oxon and parathion as shown in table 6. It is evident that the two phosphonates are highly effective insecticides roughly equal or slightly superior in toxicity to para-oxon and parathion under conditions of the tests made. The two P=O compounds III and para-oxon were much less effective as mosquito larvicides than the P=S compounds XXVI and parathion which have much greater water stability (tables 2 and 3).

Table 6.—Comparative toxicities of phosphonate- and phosphate- esters to several insects.

Compound	*M. domestica* LD_{50} γ/gm.	*P. americana* LD_{50} γ/gm.	*C. 5-fasciatus* (l.) LC_{50}— p.p.m.	*H. haemmorhoidalis* Approximate LD_{50}	*P. citri* (%)	*E. acrea* (l.) Dosage vs. Mortality (mg.)	(%)
O-ethyl *O*-*p*-nitrophenyl propylphosphonate (III)	2.0	0.70	0.052	0.00001	0.0006		
para-oxon	1.2	.75	.034	.00015	.0015		
O-ethyl *O*-*p*-nitrophenyl ethylphosphonothionate (XXVI)	0.92	.80	.0055			1	100
						0.3	60
						0.1	20
parathion	.92	1.2	.0073	.0001	.001	1	70
						0.1	0

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Oral Toxicity Studies with Heptachlor and Heptachlor Epoxide in Young Calves[1]

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ABSTRACT

Oral toxicity studies using 13 dairy calves, 1 to 2 weeks old, showed that heptachlor epoxide was approximately 10 times as toxic as heptachlor.

Both heptachlor and heptachlor epoxide accumulated to produce poisoning when given in multiple doses. A dosage level of 0.2 mg./kg. of heptachlor epoxide for 100 consecutive days (totaling 20 mg./kg.) produced no harmful effects in a dairy calf.

The symptoms and lesions of heptachlor and heptachlor epoxide poisoning were identical to those exhibited by other cyclodiene chlorinated hydrocarbon insecticides.

Residues of heptachlor epoxide were found in the fat of all the calves treated with heptachlor or its epoxide. However, chemical analyses for heptachlor epoxide in fat were not a means of diagnosing heptachlor or heptachlor epoxide poisoning.

Heptachlor is used extensively against soil and crop insects. It belongs to the cyclodiene group of chlorinated hydrocarbon insecticides. Chemically it is, 3*a*,4,5,6,7,8,8-heptachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanoindene. Other commonly known insecticides belonging to this group include chlordane, toxaphene, aldrin, and endrin.

When taken into the animal body, heptachlor is metabolized and stored in the fat as heptachlor epoxide (Metcalf 1955). Chemically, heptachlor epoxide is 1,4,5,-6,7,8,8-heptachloro-2, 3-epoxy-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene. Recent findings (Gannon & Bigger 1958, Gannon & Decker 1958) also show a residue of heptachlor epoxide may be formed on foliage and in soil after heptachlor has been applied.

Radeleff *et al.* (1955) found that the maximum nontoxic dose of heptachlor in 1- to 2-week-old dairy calves was between 15 and 25 mg./kg. body weight.

OBJECTIVES. The objectives of these studies were: 1) to compare the relative acute toxicity of heptachlor epoxide to heptachlor; 2) to determine the accumulative toxicity of heptachlor and heptachlor epoxide, 3) to observe the symptoms and lesions produced by these compounds, 4) to determine whether the amount of heptachlor epoxide found in the body fat correlated with the amount of insecticide given, or with the severity of poisoning.

MATERIALS AND METHODS.—The insecticides used in

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these studies were technical heptachlor and heptachlor epoxide of 99.5% purity, supplied by the Velsicol Chemical Corporation.

All formulations were emulsions prepared from an emulsifiable concentrate composed of 25% insecticide, 65% xylene and 10% Triton X-100 by weight.

The animals used were 1- to 2-week-old dairy calves purchased from a local auction. All were in good health when the insecticides were initially administered.

The insecticides were administered orally with a dose syringe. After dosing, each animal was observed constantly during the daylight hours and periodically at night.

Necropsies were performed on those that died, and the renal fat was preserved for heptachlor epoxide analysis. The samples were analyzed using the method described by Meyer *et al.* (1958) but deviated slightly by using alumina in the chromatographic clean-up procedure instead of Florex.

RESULTS.—*Heptachlor Epoxide, Single Doses.*—Six calves were given single doses of heptachlor epoxide.

Two were given 25 mg./kg. and three were given 15, 10, and 5 mg./kg. body weight, respectively. All were severely poisoned and died within 3 hours to 3 days. A sixth calf, which received 2.5 mg./kg., was very mildly poisoned and recovered.

Heptachlor Epoxide, Multiple Doses.—Four calves were given multiple doses of heptachlor epoxide.

The first was given 15 mg./kg. body weight without exhibiting symptoms, but when the dose was repeated 5 days later it died within 8 hours.

Another calf received 3.5 mg./kg. without presenting symptoms. For 3 consecutive days, thereafter, it was given 2.5 mg./kg. Symptoms were exhibited on the third day, but death did not occur until 7 days after the initial treatment, or 3 days after the last treatment.

A third calf received 1 mg./kg. per day for 15 days. No symptoms were presented until the last day of treatment, when it suddenly went into convulsions and died.

A fourth calf was given 0.2 mg./kg. per day for 100 consecutive days. No symptoms were exhibited, and the calf thrived throughout the test.

Heptachlor, Multiple Doses.—Three calves were given multiple doses of heptachlor.

One calf received three consecutive daily doses of 10 mg./kg. Mild symptoms were presented on the second day of treatment and became pronounced on the third and ensuing days. Death occurred 3 days after the third and last treatment, or 6 days after the initial treatment.

A second calf received 5 mg./kg. for 6 days, and a third received 2.5 mg./kg. for 15 days. Both these calves exhibited pronounced symptoms and died a few hours after their last respective doses were given.

Table 1 summarizes these results.

DISCUSSION OF ACUTE TOXICITY RESULTS.—These results show that heptachlor epoxide is approximately 10 times as toxic to young calves as technical heptachlor. The maximum nontoxic oral dose of heptachlor epoxide appears to be between 1 and 2.5 mg./kg. body weight, compared with 15 to 25 mg./kg. for heptachlor.

The results show that heptachlor epoxide is accumulative when given in multiple sublethal doses. Repeated doses of as little as 1 mg./kg. will accumulate to produce poisoning. However, the fact that a calf received 0.2 mg./kg. daily for 100 days, with no untoward effects, indicates that this dosage is, or approaches, that at which cumulative toxicity no longer exists. This calf received a total of 20 mg./kg. heptachlor epoxide and was unaffected while the three that were given 15, 2.5 and 1.0 mg./kg. for 2, 4, and 15 days, respectively, received a total of 30, 11, and 15 mg./kg., and death resulted.

The multiple-dose toxicity studies with technical heptachlor demonstrated its accumulative effect. The two calves that received 10 mg./kg. and 5 mg./kg. daily,

Table 1.—Results of toxicity studies with heptachlor epoxide and heptachlor in 1- to 2-week old dairy calves.

Ear tag Number	Dose (mg./kg.)	Number of Daily Doses	Weight of Animals, (kg.)	Total (mg.) Given	Total mg./kg. Given	Interval, Initial Treatment to Death	Heptachlor Epoxide in Fat (p.p.m.)
			Heptachlor Epoxide				
2234	25.0	1	45.0	1138	25	3 days[a]	66.7
2235	25.0	1	41.0	1025	25	4 hours	2.7
2239	15.0	1	31.8	480	15	4 hours	2.8
2238	10.0	1	32.7	327	10	28 hours	250.0
2243	5.0	1	39.5	198	5	3 hours	7.9
2267	2.5	1	50.0	125	2.5	Mildly poisoned and recovered	No sample
2236	15.0	2[b]	41.9	1255	30.0	6 days	48.3
2249	2.5[c]	4	50.0	550	11.0	7 days	93.0
2269	1.0	15	33.6	504	15.0	15 days	262.0
2268	0.2	100	—[d]	1205	20.0	No symptoms	14.7, 2.6[e]
			Heptachlor				
2242	10.0	3	37.3	1119	30.0	6 days	62.0
2244	5.0	6	42.0	1260	30.0	6 days	38.0
2248	2.5	15	38.6	1447	37.5	15 days	74.0

[a] Calf was treated with chloral hydrate when symptoms appeared the first day.
[b] Doses were given 5 days apart.
[c] The first dose given was 3.5 mg./kg.
[d] Calf weighed 39.1 kg. initially; 95.5 kg. at termination—Average daily gain 0.56 kg.
[e] Two omental fat samples were taken by biopsy; one the last day of treatment, the second 43 days later.

respectively, required an accumulative total of 30 mg./kg. to cause death. The calf that received 2.5 mg./kg. daily died after having taken a total of 37.5 mg./kg.

SYMPTOMS AND LESIONS.—The symptoms exhibited by both the heptachlor- and heptachlor epoxide-affected calves were typical of those produced by other cyclodiene chlorinated hydrocarbon insecticides (Radeleff 1958, Radeleff & Bushland 1950, Radeleff & Woodard 1955, Radeleff *et al.* 1955). Central nervous stimulation was invariably observed, which was manifested early by muscle spasms about the head and neck. These spasms progressed posteriorly and increased in severity, resulting in tonic-clonic convulsions and finally death. During convulsive seizures, the body temperature often became as high as 113° to 116° F. Frequently these seizures were interrupted by periods of depression, which lasted from a few hours to 2 to 3 days, but death always occurred during convulsions.

The lesions observed were no different from those produced by other cyclodiene chlorinated hydrocarbon insecticides. Dark, congested lungs, epicardial hemorrhages, and engorged brain blood vessels were most often presented. Frequently the gastric mucosa appeared parboiled probably owing to the extreme body temperature. Occasionally the entire gastro-intestinal tract was hyperemic and hemorrhagic.

CHEMICAL ANALYSES.—Results of the analyses for heptachlor epoxide in the renal fat are included in table 1 and show that the amount of heptachlor epoxide found in renal fat was not necessarily an indication of the severity of poisoning or the dosage given, but correlated with the time interval from initial exposure to death. Those calves that presented symptoms, and died within a few hours after being drenched, stored the least amount in their fat. The three calves that received heptachlor epoxide at 25, 15, and 5 mg./kg., respectively, and died within 4 hours, stored only 2.7, 2.8 and 7.9 p.p.m. in their renal fat.

On the other hand, the two calves that received 25 and 10 mg./kg., but lived for 3 days and 28 hours, respectively, contained 66.7 and 250.0 p.p.m. heptachlor epoxide in their renal fat. The fact that the calf treated with 10 mg./kg. contained nearly four times as much heptachlor epoxide in its fat as the calf treated with 25 mg./kg. may appear to be a discrepancy. However, this deviation is due in large part to the fact that the calf receiving 10 mg./kg. possessed very little body fat, causing the heptachlor epoxide to be more concentrated in the small amount of fat present.

Of the four calves given multiple doses of heptachlor epoxide at 15, 2.5, 1.0 and 0.2 mg./kg. for 2, 4, 15, and 100 days, the first three lived 6 to 15 days after their initial treatment. Their fat contained 48 to 262 p.p.m. heptachlor epoxide. The fourth calf, which received 0.2 mg./kg. for 100 days (a total of 20 mg./kg.), but remained normal, was subjected to two omentectomies (Radeleff 1950); one on the last day of treatment, the other 43 days later. The omental fat taken on the last day of treatment contained 14.7 p.p.m., and the fat taken 43 days later contained 2.6 p.p.m. heptachlor epoxide.

The three calves receiving technical heptachlor at 10, 5, and 2.5 mg./kg. for 3, 6, and 15 days, respectively, lived from 6 to 15 days after their first treatment. Their renal fat contained 38 to 74 p.p.m. heptachlor epoxide.

These results show that the fat of three calves that died acutely of poisoning contained less than half as much heptachlor epoxide as the fat taken from the calf treated for 100 days at 0.2 mg./kg., but showing no symptoms of poisoning.

Those calves that lived more than 1 day after initial exposure, or were given multiple sublethal doses of insecticide, and eventually died of poisoning, contained from 5 to 100 times as much heptachlor epoxide in their fat as those that died within 4 hours, even though their dosage levels were no greater. It is logical that those calves dying within a few hours would not have sufficient time to accumulate as much heptachlor epoxide in their fat as those living for several days after initial exposure.

When the dosage of heptachlor or its epoxide is low enough to be given for an extended period without producing symptoms, the level of intake influences the amount of heptachlor epoxide storage in the body fat. This is demonstrated by the calf receiving 0.2 mg./kg. heptachlor epoxide for 100 days (totaling 20 mg./kg.) with no ill effects. The fact that the omental fat of this calf contained 14.7 p.p.m. heptachlor epoxide at the end of treatment indicates that this dosage level limited its storage in the fat.

Similar unpublished results were obtained from studies at the U. S. Department of Agriculture Laboratory, Kerrville, Texas, in which yearling Hereford cattle were fed heptachlor and heptachlor epoxide at varied levels in their feed for 8 to 16 weeks. Periodic analyses showed that the amount of heptachlor epoxide storage in their omental fat was influenced by the intake level of insecticide in the feed.

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Rice Weevil Damage to Stored Corn[1]

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ABSTRACT

One of the most destructive insect pests of stored corn in North Carolina is the rice weevil, *Sitophilus oryza* (L.). No accurate figures on the amount of damage caused by this pest under North Carolina conditions have been available. This study attempts to determine not only the weight losses occasioned by the weevil but also the loss in nutritive value that results from weevil attack. Weevil damage and weight loss were determined in single kernels of corn as well as in lots of 20 and 250 grams stored at 60°, 70°, and 80° F. Nutritive losses were determined in 20- and 250-gram lots of corn by chemical analysis and by feeding tests on mice.

The total loss in weight of corn was directly proportional to the number of weevils in the corn. The greatest loss occurred at 80° F. in each of the storage tests. In the single-kernel test, weevils were responsible for loss in weight of 74.7%; in the 20-gram test they caused a loss of 12.9%, and in the 250-gram test 25.9%. The protein content of the corn increased with a corresponding increase in weevil infestation. Mice ate less and showed the least gain in weight on meal prepared from heavily infested corn.

The most common insect pest of stored corn in North Carolina is the rice weevil, *Sitophilus oryza* (L.). General estimates of the amount of damage caused by the rice weevil in the State are not thought to be very accurate. The study reported here is an attempt to provide more accurate information than has heretofore been available on the actual weight loss and damage caused by weevils in different quantities of shelled corn stored at 60°, 70°, and 80° F. and on the nutritive value of weevil-infested corn as determined by chemical analysis and by feeding tests with mice.

Information on actual weight loss in stored seeds under laboratory conditions was reported for wheat by White (1953) and by Gerberg & Goldheim (1957) for popcorn and cowpeas. White found a 30% loss of weight in one kernel of wheat caused by rice weevil feeding after 5 weeks of storage. Goldberg and Goldheim noted that the bean weevil, (*Acanthoscelides obtectus* (Say)) caused a weight loss of 68.7% per bean after 60 days of storage. The average weight loss caused by one bean weevil in one bean in 60 days was 3.5%.

METHODS AND MATERIALS.—Four tests were used to study the weight and nutritional losses in corn caused by the rice weevil. In the first, single kernels of corn were infested with weevils. These kernels were selected from a stock culture of 70 grams of corn that had been stored with 50 weevils for 2 days. Each infested kernel was kept in an individual covered pillbox, $\frac{7}{8}''$ deep and $2\frac{3}{8}''$ in diameter. The same type of pillbox was used in test 2, where each sample consisted of 20 grams of corn stored with 10 weevils. In test 3, lots of 250 grams of corn with 40 weevils were stored in covered glass fruit jars $3\frac{1}{2}''$ in diameter and 4″ deep. Rice weevils in all tests were 10 days old at the beginning of the test. The sexes were not kept separate. In test 4, infested corn from the 250-gram test was used in feeding experiments on mice.

Only one hybrid corn was used in all these tests—Dixie 82, a popular hybrid in many eastern North Carolina counties. Corn was stored at temperatures of 60°, 70°, and 80° F. and at a relative humidity of 50%. The moisture content of the grain itself was approximately 12% at the start of the test as determined by a Tag-Heppenstal moisture meter.

The losses in weight of corn in tests 1 and 2 were determined at 2-week intervals from the 4th week up to the 14th week of storage. In test 3 weight losses were calculated at monthly intervals starting at 1 month and extending to 4 months after storage. Frass, insect debris, excrement, and dead and live weevils were removed from the grain by screening through a 14-mesh screen. The grain was then weighed and this weight was adjusted for loss of moisture. The loss of moisture in the check grain was used as a correction factor for adjusting the loss in weight in the infested samples. After each weighing the live weevils were returned to the proper grain sample.

In tests 2 and 3, subsamples of grain were drawn from each treatment for chemical analysis after each weighing. Weevils inside the grain were killed by fumigation for 12 hours with calcium cyanide. No attempt was made to remove the hidden internal infestation before the corn was fumigated.

Corn from test 3 after 4 months of storage was used as the basis of the diets fed mice in test 4. Weevils in this grain were killed by heating the corn to 140° F. for 24 hours, after which the loose internal excreta, weevil fragments, and debris were removed by screening. The corn was ground in a No. 1 Wiley Mill, and the meal was passed through a 2-mm. sieve. It was not possible to remove all the internal weevil infestation prior to the grinding of the grain.

Four different diets were prepared from this corn. Uninfested corn meal was designated as diet I, and meals prepared from corn held at 60°, 70°, and 80° F. were designated as diets II, III, and IV, respectively. The meal in each diet had supplemental ingredients added as follows:

	Per Cent by Weight
Drackett assay protein C-I	6
Wesson oil	5
Vitaminized cerelose	5
Cod liver oil	1
Wesson salt	3
Added to corn, ground	80

Female Swiss albino mice (*Mus musculus musculus*), about 3 weeks old, were randomly assigned in lots of 10 to each diet. The mice were kept in individual cells and weighed at 7-day intervals until the test terminated at the end of 25 days.

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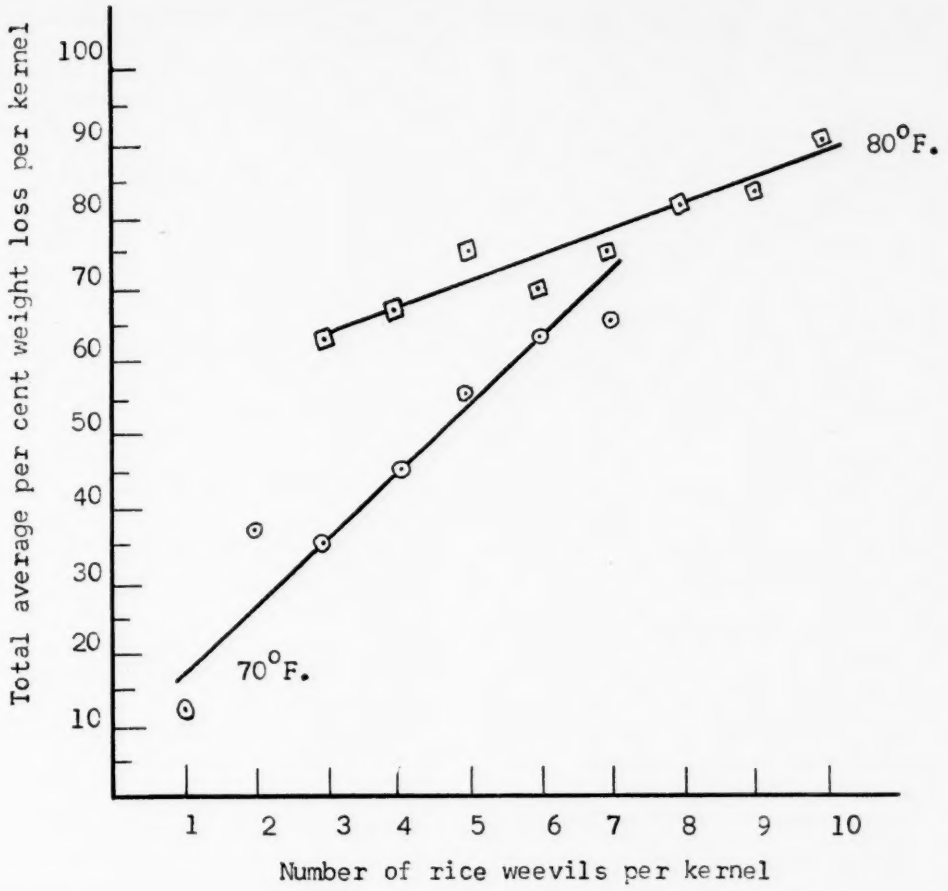

FIG. 1.—Weight loss in relation to number of weevils per kernel in single-kernel test after 14 weeks of infestation at 70° F. and 80° F.

RESULTS OF STORAGE TESTS.—In test 1, where only a single kernel of corn was used, weight losses at 60° F. were negligible after 10 weeks of storage. Weight losses at 70° and 80° F. were considerable and these are presented in table 1 and figure 1. At 70° F. the average weight loss sustained in the fourth week of storage was 7.0%, and after 8 weeks of storage the weight loss was 16.0% of the original weight. After the 14th week the total weight loss was 39% of the original weight. The average weight loss caused by one weevil after the 14th week of storage was 11.6%.

In this test (at 70° and 80° F.) the number of weevils increased with length of storage as did the total weight loss of the corn (expressed in milligrams or as per cent loss of the original weight; see table 1 and figure 1). However, the weight loss caused by one weevil (per cent weight loss divided by the number of emerged weevils) became less as the storage period progressed. For example, after 8 weeks of storage at 70° F. the average per cent weight loss per weevil was 40 and the average number of weevils was 0.4, but after 14 weeks of storage the average per cent weight loss per weevil was 11.6 and the average number of weevils was 3.4.

At 80° F., the development of the weevils was faster than that at 70° F. Weevil emergence was noticed after the fourth week of storage, 2 weeks earlier than at 70° F.

Table 1.—Average number of weevils and average weight loss per single kernel of corn at 4 to 14 weeks after storage at 70° and 80° F.

CRITERIA	70° F.						80° F.					
	Weeks of Storage						Weeks of Storage					
	4	6	8	10	12	14	4	6	8	10	12	14
Average No. of weevils	—	—	0.4	0.8	1.9	3.4	—	0.3	0.7	2.2	4.6	6.0
Average total weight loss[a]	22.0	37.6	50.1	82.6	104.3	124.9	18.6	28.8	50.3	152.6	204.2	227.7
Weight loss (%)	7.0	12.5	16.0	26.1	33.0	39.0	6.1	9.4	16.5	50.0	66.9	74.7
Average weight loss per weevil (%)			40.0	32.6	17.4	11.6	—	31.3	23.6	22.7	14.5	12.5

[a] In milligrams. Average weight per 1 kernel, 316.8 mgs.

Table 2.—Weight loss of corn in 20-gram corn samples[a] at 4 to 14 weeks after storage at 60°, 70°, and 80° F.

Weeks of Storage	Temperature (° F.)	Total Average				
		Weight Loss (Grams)	Loss (%)	No. of Weevils	Loss per Weevil (Grams)	Loss per Weevil (%)
4	60					
	70	0.8468	4.23	6.4	0.1323	0.66
	80	0.8727	4.36	7.0	0.1247	0.62
6	60	0.3202	1.60	5.9	0.0543	0.27
	70	0.8635	4.32	5.7	0.1515	0.76
	80	0.9323	4.66	9.5	0.0981	0.49
8	60	0.3728	1.86	3.0	0.1243	0.62
	70	0.9206	4.60	8.8	0.1046	0.52
	80	1.1884	5.94	12.7	0.0936	0.47
10	60	0.4268	2.13	2.8	0.1524	0.76
	70	1.0035	5.02	12.7	0.0790	0.39
	80	1.4682	7.34	18.9	0.0777	0.39
12	60	0.4816	2.41	2.3	0.2094	1.05
	70	1.0542	5.26	13.4	0.0787	0.39
	80	1.8008	9.00	23.6	0.0763	0.38
14	60	0.5042	2.52	1.8	0.2801	1.40
	70	1.1414	5.70	15.2	0.0751	0.38
	80	2.5801	12.90	39.1	0.0660	0.33

[a] Originally infested with 10 rice weevils per sample.

Table 4.—Biweekly chemical analysis of the 20-gram corn samples.

Weeks of Storage	Temperature (° F.)	Dry Matter (%)	Ash (%)	Crude Protein (%)	Crude Fat (%)	Crude Fiber (%)	N.F.E.[a] (%)
4	60						
	70	91.69	1.58	10.63	4.60	1.82	73.06
	80	91.32	1.47	10.81	4.50	1.80	72.74
6	60	89.90	1.29	10.06	4.36	1.64	72.64
	70	90.79	1.38	10.00	4.49	1.76	73.16
	80	90.49	1.34	9.94	4.42	1.72	73.07
8	60	90.18	1.22	9.88	4.44	1.99	72.65
	70	91.18	1.29	10.50	4.83	1.74	72.82
	80	90.62	1.39	10.38	4.21	1.68	72.96
10	60	90.58	1.15	10.56	4.56	1.62	72.68
	70	90.07	1.19	10.00	4.49	1.64	72.75
	80	90.02	1.45	11.81	5.92	2.32	68.52
12	60	90.27	1.19	10.13	4.55	1.67	72.73
	70	90.87	1.27	10.25	4.61	1.53	73.21
	80	89.64	1.32	10.75	4.61	1.81	71.15
14	60	91.11	1.52	10.00	4.23	1.63	73.73
	70	90.72	1.48	10.38	4.70	1.69	72.47
	80	89.06	1.71	11.19	5.33	2.52	68.31
Control[b]		89.01	1.28	9.93	4.50	1.78	71.45

[a] Nitrogen-free Extract.
[b] Average of two samples.

After 14 weeks of storage, nearly twice as many weevils emerged at 80° F. as at 70° F.

The results of test 2 are shown in tables 2 and 6. In this test 10 weevils were added to each lot of 20 grams of corn before the beginning of storage. After 14 weeks of storage the average number of weevils per sample of grain was 1.8, 15.2, and 39.1 in the 60°, 70°, and 80° F. tests, respectively. The corresponding weight losses in the grain were 0.5042, 1.1414, and 2.5801 grams, or a percentage loss in weight of 2.5, 5.7, and 12.9 at 60°, 70°, and 80° F., respectively. The average weight loss per weevil at these three temperatures was 0.2801, 0.0751, and 0.0660 gram.

In test 3, each lot of 250 grams of corn had 40 live rice weevils added to it before storage. After 1 month of storage at 60° and 70° F. less than 30 weevils per sample were present, but at 80° F., there was an average of 47.2 weevils, a gain of 19.5% over the original population of 40 weevils (see table 3). At 70° F. the original population was not surpassed until after 2 months of storage. At 60° F. the original population showed a decline for 2 months after storage, a slight rise at 3 months, and eventually at 4 months after storage the original population was surpassed.

Chemical analyses of the grain were made biweekly in the 20-gram test and monthly in the 250-gram test. In both tests the amount of crude protein increased as the period of storage increased (see tables 4 and 5). This increase appeared to be caused by the greater number of weevils present within the grain. These weevils were not removed by the process of screening, and consequently they were ground along with the grain and included in the protein analyses of the meal.

The increase in protein was more evident in the grain of the 250-gram test. One month after storage the protein content at 60°, 70°, and 80° F. was, respectively, as follows: 8.81%, 8.88% and 9.06%. After 2 months of storage the protein content increased at 70° and 80° but not at 60° F., where the protein content remained at its original level, 8.81%. After 4 months of storage the pro-

Table 3.—Weight loss per weevil in 250-gram samples[a] of corn at 1 to 4 months after storage at 60°, 70°, and 80° F.

Months of Storage	Temperature (° F.)	Total Mean Loss (Grams)	Total Loss (%)	Average No. of Weevils	Loss per Weevil (%)
1	60	2.00	0.80	27.8	0.029
	70	2.34	.94	25.8	.036
	80	3.85	1.54	47.2	.033
2	60	2.05	0.82	22.0	0.037
	70	3.61	1.44	47.0	.031
	80	13.33	5.33	283.8	.019
3	60	2.95	1.18	35.0	0.034
	70	8.28	3.31	111.6	.030
	80	33.80	13.52	453.6	.030
4	60	6.24	2.50	44.2	0.056
	70	17.06	6.82	210.8	.032
	80	64.73	25.89	626.8	.041

[a] Originally infested with 40 rice weevils per sample.

Table 5.—Monthly chemical analysis of the 250-gram corn samples.

Months of Storage	Temperature (° F.)	Dry Matter (%)	Ash (%)	Crude Protein (%)	Crude Fat (%)	Crude Fiber (%)	N.F.E.[a] (%)
1	60	89.99	1.50	8.81	4.22	1.46	74.00
	70	89.99	1.46	8.88	4.26	1.50	73.89
	80	88.88	1.45	9.06	4.33	1.48	72.56
2	60	89.05	1.66	8.81	3.98	1.55	73.05
	70	89.04	1.64	9.38	3.47	1.65	72.90
	80	82.74	1.71	9.25	1.22	1.79	68.77
3	60	90.80	1.44	8.44	4.22	1.58	75.12
	70	88.07	1.64	8.88	4.31	1.64	71.60
	80	84.85	1.83	9.44	2.84	1.86	68.88
4	60	95.39	1.34	9.00	4.17	1.45	79.43
	70	96.38	1.66	10.69	3.82	1.62	78.59
	80	96.60	1.84	12.38	2.82	2.13	77.43
Control		90.26	1.50	8.50	4.30	1.36	74.60

[a] Nitrogen-free Extract.

tein content increased to 9%, 10.69%, and 12.38% at 60°, 70°, and 80° F., respectively. It was estimated that at 80° F. the protein content of 250 grams of uninfested corn at this time was 18.71 grams, and of the infested corn 19.67 grams.

RESULTS OF MICE DIET TESTS.—The mice fed on diets I, II, and III showed no significant differences in weight gained during the 25-day feeding period. Mice fed diet IV (weevil-infested corn stored at 80° F.) showed a marked decrease in weight in the same period. The mean

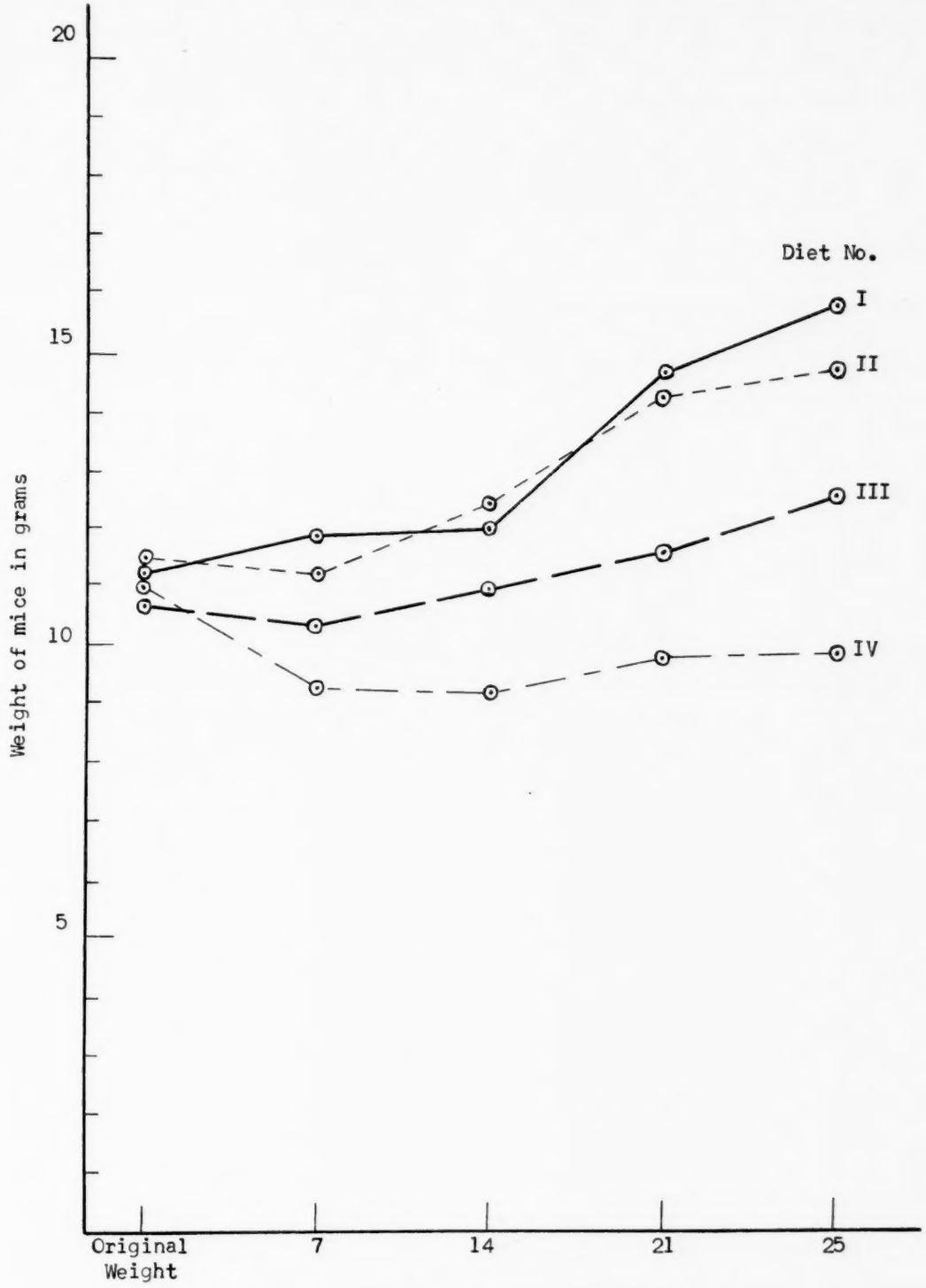

FIG. 2.—Rate of growth of mice on four different diets of corn meal.

Table 6.—Per cent weight losses and weevil population in single kernel, 20-gram, and 250-gram tests.

Storage in Weeks	Single Kernel Test		Average Loss per Weevil (%)					
			20-Gram Test			250-Gram Test[a]		
	70° F.	80° F.	60° F.	70° F.	80° F.	60° F.	70° F.	80° F.
4			—	0.66	0.62	0.029	0.036	0.033
6		31.3	0.27	.76	.76			
8	40.0	23.6	.62	.52	.47	.037	.031	.019
10	32.6	22.7	.76	.39	.39			
12	17.4	14.5	1.05	.39	.38	.034	.030	.030
14	11.6	12.5	1.40	.38	.33	.056	.032	.041
	Average number of weevils per sample							
4				5.7	7.0	28.8	25.8	47.1
6		0.3	5.9					
8	0.4	0.7	3.0	8.8	12.7	22.0	47.0	283.8
10	0.8	2.2	2.8	12.7	18.9			
12	1.9	4.6	2.3	13.4	23.6	35.0	111.6	453.6
14	3.4	6.0	1.8	15.2	39.1	44.2[b]	210.8[b]	626.8

[a] Weighing was done every month.
[b] Average in the 4-month period.

loss in weight for the mice fed diet IV was 1.442 grams, whereas mice in diets I, II, and III had an average gain in weight of 4.58, 3.233, and 1.887 grams, respectively. It may be noted that although the gains in weight of mice fed diets I, II, and III were not significantly different, nevertheless, a decreasing rate of weight increase was evident in the test mice corresponding to an increase in the original weevil infestation in corn used for these diets.

The rate of change in mouse body weight fed different diets is presented in figure 2. A decrease in body weight occurred after 7 days of feeding in all mice except those fed diet I. The decrease was most marked in diet IV. Mice on this diet showed very little recovery in weight at the end of 25 days, whereas mice fed the other diets showed an increase in weight from the seventh day of their diets to the end of the 25-day feeding period. In general, gain in weight of mice was proportional to the quality of the corn. The mean gain or loss in mouse body weight is shown in figure 3.

Based on the total feed intake by mice, a significant difference was noted between diets I and IV, and between diets II and IV at the 5% level. No significant difference occurred at the 5% level between diets I and II or between diets III and IV.

The difference in weight gained by the mice is not believed to be due to the different amount of protein in the feed, but rather to the differences in the amount of feed

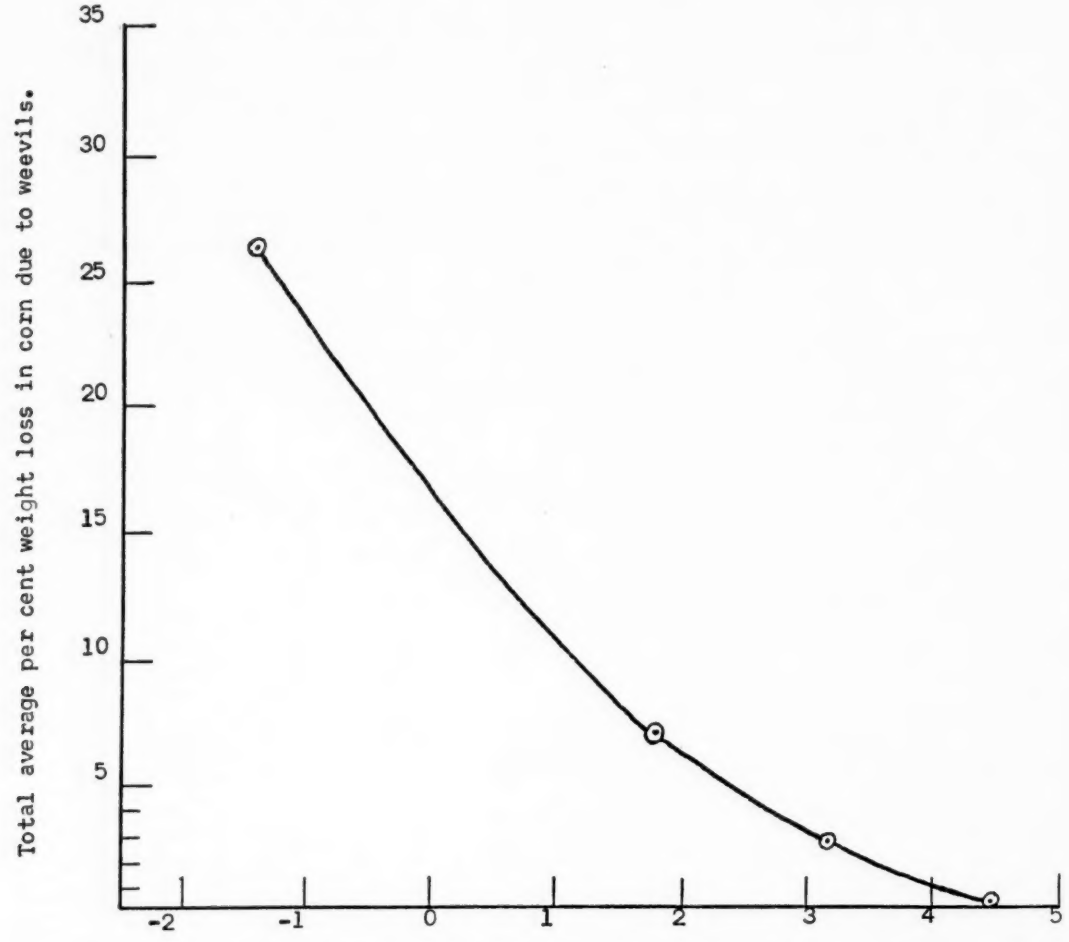

FIG. 3.—Mean loss or gain in weight (in grams) of mice after 25 days of feeding in relation to the average per cent loss in weight of stored corn caused by the rice weevil.

Table 7.—Comparative efficiency of the four diets fed to mice in 25-day period.

	Diet Number							
	I		II		III		IV	
	Total		Total		Total		Total	
Mice No.	Feed Intake (Gm.) X	Gain Weight (Gm.) Y	Feed Intake (Gm.) X	Gain Weight (Gm.) Y	Feed Intake (Gm.) Y	Gain Weight (Gm.) Y	Feed Intake (Gm.) X	Gain Weight (Gm.) Y
1	93	2.8	55	2.0	89	4.5	—	—
2	89	8.2	91	5.8	—	—	64	3.5
3	70	6.7	67	−2.5	47	2.1	29	−3.7
4	77	7.4	—	—	76	7.7	33	−4.0
5	74	6.5	94	6.1	46	−1.7	63	3.9
6	82	3.2	65	1.2	36	1.3	—	—
7	82	6.3	66	5.3	48	−3.6	—	—
8	37	−3.9	53	2.4	—	—	54	−0.2
9	77	9.3	55	3.8	92	4.7	46	−5.2
10	56	−0.7	87	5.1	56	0.1	38	−4.4
Total	737	45.8	633	29.2	500	15.1	327	−10.1
Average	73.7	4.58	70.33	3.233	62.50	1.887	46.71	−1.442

Analysis of covariance

Source of Variation	Degrees of Freedom	Sums of Squares and Products			Errors of Estimate		
		Sx	Sxy	Sy_2	D.F.	S.S.	M.S.
Treatment	3	3,398.50	1,675.34	328.7505			
Error	30	8,921.53	360.65	405.5460	29	360.9669	13.4816
Total	33	12,320.03	2,035.99	734.2965	32	397.8318	12.4322
Differences for testing adjusted means					3	6.8649	2.2883
F value = 0.1697[a]							

[a] Not significant at 95% fiducial limits.

intake. It may be assumed that if the mice ate the same amount of feed their growth rate would be more or less the same. The covariance analysis of feed intake and mouse-weight gain indicates that there is no significance between feed intake of the different diets and the gain in mice weight. The F-value on the adjusted treatment mean was 0.1697 whereas an F-value at 5% level of 2.93 is required for significance (see table 7).

DISCUSSION.—In all three infestation tests (single-kernel, 20-gram, and 250-gram), there was a marked development of weevils and subsequent weight loss at 70° and 80° and the least at 60° F. At 70° F. in the 20-gram test the population had increased 50% (from 10 to 15.2 weevils per sample). In the 250-gram test the increase in the same period at 70° F. was more than 500% (from 40 to 210.8 weevils per sample). At 80° F. the increases in population after 14 weeks of storage were even more marked than at 70° F., reaching almost a 391% increase in the 20-gram test (from 10 to 39.1 weevils per sample) and more than 1,500% in the 250-gram test (from 40 to 626.8 weevils per sample).

Corn stored at 80° F. sustained the greatest loss in weight. In 4 weeks of storage the 20-gram sample lost 0.8727 gram (4.4%). After the 14th week of storage the loss in weight was 2.5801 grams or 12.9%. Weight loss per weevil was found to decrease as the number of weevils increased. This condition was probably caused by the fact that less and less food was available for weevil feeding as their numbers increased. The exception to this would seem to be in the 20-gram test at 60° F., where the average weight loss per weevil increased from 0.17 gram at 6 weeks to 1.40 grams at 14 weeks. The population in this test decreased in the same period from 5.9 to 1.8 weevils.

In the single-kernel test as many as 10 weevils emerged from one kernel of corn. The average total loss in weight at 80° F. in a single kernel of corn was 74.7% after 14 weeks of storage. Comparable total weight losses in the 20-gram and 250-gram tests were 12.9 and 25.89%, respectively. These figures indicate the wide range in weight loss that is possible, dependent upon initial infestation, amount of corn, and length of storage.

A further indication of the effect of rice weevils on quality of corn was indicated by the chemical analysis of the corn and by the results of feeding different corn diets to mice. Chemical analysis of infested corn showed that protein content tended to increase with the length of storage and the increase in weevil population. This increase in protein may be caused by the increased hidden weevil infestation of the corn.

The proportion of the fiber to carbohydrate in corn was greater in heavily weevil-infested corn than it was in uninfested corn. Weevils, as Fraenkel (1943) has pointed out, prefer to feed in the endosperm of the grain. In heavily infested corn much of the endosperm is removed by weevil feeding. The result is a higher proportion of crude fiber to carbohydrate. Corn with a high content of crude fiber is no doubt less attractive as food for weevils than uninfested corn. For the same reason, heavily infested corn may be less attractive as food for mice.

When mice were fed diets made from uninfested and infested corn their gain in weight and the amount of food consumed was directly proportional to the amount of weevil infestation in the grain. This fact would seem to indicate that heavily infested corn was less attractive to mice than uninfested corn. If the assumption can be made that this conclusion also holds true for other animals, such as the usual corn fed farm livestock, then the feeding of infested grain is not a desirable practice.

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Experiments with Insecticides for the Control of Drosophila Breeding[1]

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ABSTRACT

In experiments under field conditions at Beltsville, Maryland, one application of the following insecticides gave good control of drosophila breeding in piles of cull tomatoes: aldrin, dieldrin, ethion, heptachlor, chlordane, methoxychlor, ronnel (Dow ET-57), malathion, dicapthon, Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl)phosphorothioate), and Dipterex® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate). Malathion, the only insecticide tested under commercial conditions, also gave good control of drosophila breeding in tomato skin piles on the Eastern Shore of Maryland.

Drosophila may breed in large numbers in cull piles of certain fruits and vegetables (Pepper *et al.* 1953, Dorst & Knowlton 1954, Bickley & Ditman 1954, Mason & Henneberry 1960). These concentrated breeding areas may be sources of infestation affecting nearby tomato fields and canning factories. Peach orchards in California have been reported as a likely source of infestation for tomato fields (Michelbacher & Middlekauff 1954). Canners in the eastern United States have reported similar observations. The control of breeding in such areas, either by sanitary measures or by application of insecticides, may be an important phase in a drosophila control program.

In 1954, 1955, and 1956 investigations were conducted at Beltsville, Maryland, to determine the effectiveness of certain insecticides for the control of drosophila breeding. In 1954 and 1955 experiments were also run under commercial conditions on the Eastern Shore of Maryland, using an insecticide spray for the control of drosophila breeding in tomato refuse from a canning factory[3]

LABORATORY EXPERIMENTS.—Two experiments were conducted in 1954 and one in 1956 with insecticides as sprays for the control of *Drosophila melanogaster* Meig. breeding in canned pumpkin. Widemouthed pint fruit jars were filled about one-third full of pumpkin that contained a small amount of mold inhibitor. The pumpkin, either before or after it was sprayed, was infested with eggs and larvae. This infesting was accomplished by placing the jars at random on a 5-foot diameter turntable that slowly revolved for about 48 hours in a room containing several thousand adults. Each jar received one application of spray from a nasal atomizer. Check jars were sprayed with water. All flies were removed from the jars after processing. The jars were then capped with cheesecloth and held for 2 weeks for fly emergence.

In the first experiment in 1954 the treatments were applied after the pumpkin had been infested with drosophila. Replications were sprayed July 22, 26, 29, and 30. In the second experiment applications were made immediately before the pumpkin was infested. The different replications were sprayed August 5, 6, 13, and 16. The following insecticides were tested in both experiments at 0.1% dilution: chlordane, heptachlor, lindane, DDT, TDE, aldrin, dieldrin, isodrin, malathion, parathion, Dipterex® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate), and Am. Cyanamid 12008 (*O,O*-diethyl *S*-(isopropylthio)-methyl phosphorodithioate). All insecticides were wettable powders except Am. Cyanamid 12008, an emulsifiable concentrate, and Dipterex, a technical-grade powder. Dosages were not measured, but the surface of the pumpkin in each jar was thoroughly sprayed.

In the first experiment all insecticides except TDE prevented drosophila breeding. A total of 3,095 adults emerged from the check jars, 364 from the jars treated with TDE, 1 from DDT, and none from any of the others. The second experiment gave essentially the same results. All prevented breeding except TDE, which gave 80% control. A total of 1,481 flies emerged from the checks and 289 from the jars sprayed with TDE.

For the 1956 experiment, jars of pumpkin were infested during the period July 16 to 18 and sprayed within a few minutes after their removal from the turntable. The insecticides tested at 0.1% concentration were malathion, dicapthon, ronnel (also known as Dow ET-57) Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate), Dipterex, Thimet® (*O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate), and piperonyl butoxide plus 0.05% of Triton X-100 (alkylated aryl polyether alcohol). Dipterex and piperonyl butoxide were technical-grade materials and Thimet was an emulsifiable concentrate. The other insecticides were wettable powders. All formulations were tested on three jars of pumpkin. Each jar received about 1 cc. of spray.

All insecticides prevented breeding except piperonyl butoxide, which reduced breeding 95%. From the check jars 2,015 adult flies emerged and 95 from those sprayed with piperonyl butoxide.

FIELD-CAGE EXPERIMENTS.—In 1954 and 1955 bait

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[2] Richard Lehr left the Division in 1958. B. K. Dozier, formerly of this Division, assisted in these tests.

[3] These experiments were conducted in cooperation with T. Noble Jarrell, Inc., Goldsboro, Maryland.

sprays were tested in cheesecloth-covered cages for the control of drosophila on tomatoes (Mason 1956). The cages were artificially infested with adults of *Drosophila melanogaster*. Quart strawberry boxes full of cull tomatoes were sprayed with a hand sprayer until there was a run-off; dosages were not measured. One box was placed on the ground in each cage within 1 hour after treatment. Records were also taken on drosophila breeding in each box by counting the pupae about 2 weeks after the sprays had been applied.

In 1954 the boxes of tomatoes used for this study were sprayed on September 14 with heptachlor, aldrin, malathion, or Dipterex at 0.1% concentration. All sprays also contained 10% blackstrap molasses, 1% apple cider vinegar (acid content 5%), and 0.1% active dry yeast (Fleischmann's type 1821). The checks were sprayed with water. The experimental design was randomized blocks with three replications. Precipitation for 7 days following treatment was 0.62 inch. Aldrin prevented breeding almost 100%; heptachlor reduced breeding 95%, Dipterex 88%, and malathion 51%. Approximately 10,036 larvae pupated in the check boxes.

In 1955 the tomatoes in strawberry boxes were sprayed on September 15. The insecticides tested were malathion, Thimet, dicapthon, ronnel, DDVP, aldrin, dieldrin, isodrin, lindane, and heptachlor. All were tested at a concentration of 0.1% except malathion, which was at 0.2%. All insecticides were wettable powders except Thimet, a powder on active carbon with 0.05% of Triton X-100 added. Each spray also contained 10% granulated sugar, 1% apple-cider vinegar (acid content 5%), and 4% active dry yeast (Fleischmann's type 1821). The check boxes were unsprayed. The experiment was completely randomized, with the treatments replicated three times. Precipitation for 7 days after treatment was 0.26 inch.

Ronnel, Thimet, aldrin, dieldrin, lindane, isodrin, heptachlor, and dicapthon prevented breeding 97% to 100%, malathion (premium grade) 88%, and DDVP 78%. The check boxes produced 3,290 pupae.

CONTROL OF BREEDING IN PILES OF CULL TOMATOES.—In all three seasons insecticides were also tested for the control of drosophila breeding in piles of cull tomatoes in the field. The piles contained 1 to 2 bushels and were 9 feet apart (fig. 1). The variety of tomatoes was Rutgers except as indicated otherwise. Sprays were applied with a 2-gallon compressed-air hand sprayer, and the one granular formulation used in 1956 was scattered by hand. Results were determined by counting drosophila pupae attached to small wooden stakes stuck in a vertical position on each pile of tomatoes. The number of stakes per pile was uniform in each experiment but varied from 5 to 14 in the different experiments.

Tomatoes for the first experiment in 1954 were picked and piled on September 22 and 24, and the piles were sprayed on September 25. The insecticides tested were malathion at 1% and Dipterex, heptachlor, chlordane, aldrin, or dieldrin at 0.5%. All were wettable powders except Dipterex, which was a technical-grade powder. Dosages applied to three piles of tomatoes ranged from 1,260 to 1,350 cc. The piles were heavily infested with drosophila adults at the time of spraying, and many of the tomatoes were infested with eggs and larvae when harvested. The experimental design was randomized blocks with three replications. Rainfall for the 7-day period immediately following treatment was 0.11 inch.

All insecticides controlled breeding 99% to 100% for more than 1 month. A total of 1,772 pupae were counted on the stakes in the check piles, as compared with 18, the highest number for any of the treatments.

Tomatoes for the second experiment in 1954 were picked and piled on October 12. They were sprayed on October 13 with methoxychlor suspension at 0.5% or di-

FIG. 1.—Piles of cull tomatoes treated with insecticides for the control of drosophila breeding.

capthon emulsion at 1%. Total dosages for three replications were 1,700 cc. for methoxychlor and 1320 cc. for dicapthon. The piles were heavily infested with drosophila flies when sprayed. A total of 1.46 inches of rain fell within 3 days after treatment.

Dicapthon controlled drosophila breeding almost 100% for 37 days or longer, and methoxychlor reduced it by 96%. A total of 1,198 larvae pupated on the stakes in the check piles. Drosophila breeding was heavy in the check piles of both experiments.

Tomatoes for the 1955 experiment were a mixture of varieties picked and piled on October 11. They were sprayed on October 13 with dieldrin or dicapthon suspension at 0.25%. Total dosages applied to four piles or replications were 1,568 and 1,498 cc., respectively. The piles were heavily infested with drosophila flies at the time of treatment. More than 4 inches of rain fell within 24 hours after the piles were sprayed. The treatments were completely randomized.

Records taken 20 and 35 days after the piles were sprayed showed that both insecticides controlled breeding almost 100%. Breeding in the checks was lighter than in 1954. A total of 305 pupae were attached to the stakes in the check piles.

In 1956 the tomatoes were picked and piled on October 10 and 11. On October 12, 300 laboratory-reared *melanogaster* adults were released on each pile. The piles were treated on October 13 with the following insecticides: sprays—malathion at 0.5% and Diazinon, dicapthon, ethion, heptachlor, and ronnel at 0.25%; granules—ronnel 5%. All sprays were prepared from wettable powders. Dosages applied to four piles varied from 1,400 to 1,820 cc. of spray and 88.4 grams of granules. The experimental design was randomized blocks with four replications. Precipitation for the first 7 days after treatment was 0.24 inch.

All treatments controlled drosophila breeding 99% to 100% for 33 days. Breeding in the check piles was heavy. A total of 1,575 larvae pupated on the check stakes, as compared with 16, the highest number for any of the treatments.

CONTROL OF BREEDING IN COMMERCIAL PILES OF REFUSE.—In 1954 and 1955 tomato refuse from a commercial cannery was sprayed by the canner to control drosophila breeding. Treatment was made with a power sprayer on October 16 in 1954 and October 20 in 1955, a day or two after canning operations were discontinued for the season. Only that portion of the pile containing fresh refuse was sprayed. The dosage was not measured either year, but the skins were thoroughly sprayed.

In 1954 one application of a 1.5% malathion emulsion completely prevented breeding for 27 days or longer. Similar results were obtained in 1955 with one application of a 0.75% malathion emulsion. In both years breeding was heavy in unsprayed skin piles in the general area that were used as checks.

CONCLUSION.—Results of these experiments indicate that drosophila breeding can be controlled in cull tomatoes by a thorough application of any one of several insecticides.

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Feeding and Oviposition by the Boll Weevil on Artificial Diets[1]

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ABSTRACT

Artificial diets are described for the adult boll weevil (*Anthonomus grandis* Boh.). Basal diets contained an enzymatic hydrolyzate of casein, glucose, corn oil, cholesterol, Wesson's salts, choline, ascorbic acid, B vitamins, water, and agar. Mixed pollen in amounts of 0.1 to 5 grams per 100 grams of the basal diet stimulated feeding and oviposition. Five generations of weevils were obtained on artificial diets without cotton-plant parts by rearing from egg to adult on a soybean protein diet and allowing the adults to feed and oviposit on a diet containing mixed pollen. Oviposition occurred when pollens from different families of plants were used. The only substance other than pollen that appreciably stimulated oviposition was wheat germ. A basal diet containing homogenates of heated cotton-plant parts, squares, flowers, germinated cottonseed, and young green seedlings also caused weevils to feed and oviposit. Tests with modifications of the basal diets are described. Substances that repelled the boll weevil when included in the basal diets were alfalfa meal, forage juice, distiller's dried solubles, yeast extract, and beef extract.

The boll weevil, *Anthonomus grandis* Boh., perpetuates itself almost exclusively on the buds and bolls of the cotton plant. However, it has been found feeding and reproducing under natural conditions on two other plants of the family Malvaceae, *Hibiscus syriacus* L. (Gaines 1934) and *Thespesia populnea* (L.) (Lukefahr 1956). When placed in cages with other plants of this family,

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adults fed on the flowers and buds (Coad 1914) and oviposition and growth from egg to adult were observed in the wine cup, *Callirrohoe involucrata* (Nutt.) A. Gray (Walker 1959). In the laboratory, weevils will feed on various plant materials, such as peanuts, apples, bananas, and other fruit, but no normal oviposition has been observed.

It was reported previously that adults fed on the standard larval diet but laid no eggs (Vanderzant & Davich 1958). When ether or ethanol extracts of squares or blooms of the cotton plant were added, several eggs were obtained from field-collected weevils that presumably were ready to lay. The larval diet, however, did not permit ovarian development, and it was therefore necessary to find a new diet for the adult. This paper reports such experiments conducted at the College Station, Texas, laboratory in 1957 and 1958.

MATERIALS AND METHODS.—*Basal Diets.*—The composition of two basal adult diets is shown below. A was used for diets to which pollen was added in 1957. B, in which the nutrients were changed so that the amounts were similar to those in the larval diet, was used in 1958.

	Grams	
	A	B
Enzymatic hydrolyzate of casein	2	3
Glucose	2	2
Corn oil (Mazola)	0.5	0.25
Cholesterol	0.1	0.05
Wesson's salts	0.5	0.75
Choline-chloride	0.05	0.1
Vitamin mixture (Vanderzant 1957)	2 ml.	1 ml.
Ascorbic acid	0.2	0.05
Agar	2	2
Water	100 ml.	100 ml.

The methods of preparing and feeding all the diets tested in 1957 were as follows: The constituents were thoroughly mixed, the pH was adjusted to 6.5, and the diet sterilized by autoclaving at 121° C. for 15 minutes. It was poured while hot into aluminum-foil dishes approximately 1 inch in diameter and ½ inch deep, about 2 ml. of diet per dish. After the diet had solidified, the dishes were dipped in molten paraffin and stored in a refrigerator until needed. Before a dish was placed in a cage the surface was punched with a hot needle to make openings through the paraffin coating to enable the weevils to feed. One dish was placed in each cage containing 20 to 30 newly emerged weevils, approximately equal numbers of males and females. Dishes were changed daily and examined for feeding and eggs. Cages were kept in an incubator at a mean temperature of 86° F. with 12 to 14 hours of light provided by an incandescent bulb.

In 1958 the diet preparation was modified to avoid changes in the test substances during heating. Instead of autoclaving, the basal mixture was heated in a water bath until the agar was dissolved. The test substances were then added so they would be heated for not longer than 10 minutes. The diets were poured immediately into sterile aluminum dishes under aseptic conditions and stored in a refrigerator. This procedure reduced microbial growth in the diet so that no decomposition was apparent at the end of the 24-hour feeding period in the weevil cages.

Pollen Diets.—The pollens were mixed with the basal diets and prepared as described above.[3]

The following pollens were collected by bees: mixed pollen was collected in the College Station area from March 27 to April 3, 1954, air dried, and stored at room temperature; a second sample was collected in April 1958, and stored in a freezer; mustard pollen from two species of wild mustard, *Sisymbrium irio* and *Lesquerella gordoni*, obtained in March 1958, and dandelion pollen, *Taraxacum* sp., collected in May 1957 in Laramie, Wyoming were stored in a freezer.

Cotton pollen, *Gossypium barbadense*, and corn pollen, *Zea mays.*, were collected by hand and stored in a freezer. Prior to storage the cotton pollen was dried *in vacuo* at 40° C.

Wheat Germ Diets.—Cold-rolled wheat germ was obtained from the producer.[4] Toasted wheat germ, prepared for use as cereal, was purchased from a local grocery store.

The wheat-germ diet differed from the basal diets and had the following composition: 4 grams of wheat germ, 2 grams of enzymatic hydrolyzate of casein, 1 gram of glucose, 0.75 gram of Wesson's salts, 0.1 gram of choline, 0.05 gram of cholesterol, 1 ml. of vitamin mixture, 2 grams of agar, and 100 ml. of water. The constituents were mixed in a blender, heated in a boiling water bath for 10 to 15 minutes, then poured in the dishes and fed as previously described.

Proteins and Protein Hydrolyzates[5].—Wheat hydrolyzate and hydrolyzed protein insecticide baits were donated by the manufacturers. Other proteins and protein hydrolyzates were purchased.

Cotton-Plant Parts.—Fresh squares (all sizes with bracts removed), blooms (petals, stamens, and style only), medium-sized leaves, cottonseed germinated for 48 to 72 hours and then squeezed from the hulls, and green seedlings that had just emerged were heated in a small amount of boiling water for 3 to 5 minutes, depending on the density of the material. Each plant part was then beaten into a thick puree in a blender, weighed to determine the amount of water added, and stored in a freezer until needed. These homogenates were heated with the rest of the diet constituents for 5 to 10 minutes. The amounts added represented 2 to 10 grams of fresh cotton plant material per 100 grams.

PRELIMINARY TESTS.—The various nutrients used in diets for cotton insects were first tested for feeding response. Each group of substances, such as proteins and amino acids, sugars, salts, vitamins, and lipides, was mixed in a water-and-agar base and tested individually. Proteins and protein hydrolyzates were found to be the most attractive, but the mortality was higher with the proteins. Since an enzymatic hydrolyzate of casein was attractive to the weevils, it was chosen as the source of amino acids and other nutrients were combined with it. In these early tests no eggs were found on the basal diet.

DIETS CONTAINING POLLEN.—Since adults feed largely on the immature anthers in the cotton bud and also in the flower, it was logical that pollen be tested in a basal diet.

[3] J. N. Weaver, Texas Agricultural Experiment Station, supplied the bee-collected mixed pollen. Mustard, dandelion and cotton pollens were supplied by the Entomology Research Division's Bee Culture Laboratory at Tucson, Arizona.

[4] Cold-rolled wheat germ, type 75, was supplied by General Mills, Inc.

[5] Wheat hydrolyzate Huron E610, was obtained from the Huron Milling Division of Hercules Powder Co. Protein hydrolyzates, Staley's Protein Insecticide Baits No. 2 and No. 7, were obtained from A. E. Staley Manufacturing Co. Soybean protein, Drackett assay C-1, was a product of the Drackett Products Co. Other proteins and protein hydrolyzates were purchased from Nutritional Biochemicals Corp.

Table 1.—Oviposition by three successive generations of Mexican boll weevils on basal diet A containing 5 grams of mixed pollen per 100 grams, 1957.

Generation	No. of Females	Number of Eggs per Female[a] in (Days)			
		30	40	50	60
		Basal diet at half concentration			
1	11	26	35 (1)	44 (1)	55 (2)
2	12	25	38	54 (1)	—
		Basal diet at full concentration			
2	11	47	69 (1)	87	97 (5)
3	19	38	50	—	—

[a] Number of dead females indicated in parentheses.

Pollen stimulated feeding, and eggs were found in the dishes. The first successful diet contained mixed pollen collected by bees in 1954, 5 grams per 100 grams. No cotton pollen was present in this mixture.

The oviposition record for three successive generations of newly emerged adults fed on a pollen diet are given in table 1. The adults of the first generation were the fourth generation of Mexican weevils reared from egg to adult on the standard larval diet. During a 2-month period they laid 601 eggs. Only 4 of the original 11 females died; the remaining 7 were reproducing and all had large eggs in their oviducts.

Eggs from these weevils were removed from the dishes of diet, washed in the usual way, and transferred to rearing vials containing the standard larval diet (Vanderzant & Davich 1958).

Following emergence, the second-generation adults were divided into two groups. The first group was fed the same diet as the first generation and oviposited approximately the same number of eggs. The second group was given a diet in which the concentration of basal nutrients was doubled. The latter group produced in excess of 50% more eggs than the first group, or a total of 1,062 in 2 months. After the third generation, two more generations of weevils were reared in the same way, or a total of five generations on artificial diets alone, with no part or extract of the cotton plant.

Two tests were conducted (not shown in tables) in which pollen was tested as the sole nutrient source. A diet composed of 5 grams of mixed pollen, 2 grams of agar, and 100 ml. of water did not promote oviposition. With a similar diet containing 20 grams of pollen, 13 females deposited only 2 eggs per female in 20 days.

Oviposition records of newly emerged females fed in basal diets A and B to which different amounts of mixed pollen were added are given in table 2. These data and those given in table 1 indicate that 2 to 5 grams of pollen per 100 grams of basal diet promotes the best oviposition. However, individual differences made it difficult to determine the best pollen concentration from such a small number of tests. Table 2 shows the differences between groups of females given the same diet. Mixed pollen collected in 1958 promoted oviposition similar to that obtained in 1954 when used at 1 gram per 100 grams of diet. This concentration of cotton pollen was also effective. During a 25-day period oviposition on 0.1 and 0.5 gram of cotton pollen per 100 grams of diet was negligible. Mustard pollen gave fair results, but oviposition on corn pollen was poor. In fact, as the concentration of corn pollen was increased, oviposition decreased. With 4 grams of corn pollen per 100 grams of diet only two eggs per female were obtained in 26 days. Dandelion pollen was particularly attractive in promoting feeding, and five females given a diet containing 0.5 gram per 100 grams of diet laid five eggs each in 24 days.

Owing to the small amounts of pollen available, studies with pollens from known species of plants were not continued.

Unfed weevils obtained from Mexico and reared from squares feed and oviposit on the artificial diets as readily as those reared on the larval diet. Mortality on all diets with pollen was low, and females survived longer than males.

Weevils collected in the field at College Station in the late summer of 1957 fed on diets containing pollen, but failed to lay any eggs. After 10 days of feeding several females were dissected. Clear egg-shaped spaces were found in the oviducts, which indicated that large eggs had been reabsorbed, but immature eggs were present. At 22 days no change was noted.

Adults newly emerged from squares in September 1957 also failed to oviposit even though they fed heavily. During 23 days of feeding only one egg was obtained in a diet

Table 2.—Oviposition by laboratory-reared Mexican boll weevils on diets containing pollen.

Kind of Pollen	Grams per 100 ml. of Basal Diet	Number of Females	Days before Oviposition	Number of Eggs per Female in—	
				30 Days	40 Days
		1957 (Basal Diet A)			
Mixed (1954)	1.0	10	10	17	31
		10	9	9	—
	2.5	9	13	10	21
		10	6	48	65
	5.0	10	6	19	35
		18	6	12	—
		10	9	24	37
	7.5	12	8	16	27
		1958 (Basal Diet B)			
Mixed (1954)	0.1	11	7	14	—
	0.5	6	9	8	—
		5	11	19	—
	1.0	7	9	24	36
	2.0	10	5	21	—
		11	7	21	—
		8[a]	7	35	—
Mixed (1958)	1.0	9	8	25	—
Cotton	1.0	7	10	39	—
Mustard	1.0	12	11	13	—
	2.0	13	10	23	—
Corn	2.0	12	11	7	—

[a] Square reared.

containing pollen. In November adults reared in the laboratory on the standard larval diet, the progeny of adults collected in the fall, were given pollen diets. Only 17 eggs were obtained from 25 females in 30 days. In November 1958 adults reared from squares again were fed a pollen diet. A group of 10 females did not start to oviposit until after 20 days of feeding. In 50 days 54 eggs were obtained, and upon dissection 3 of the females were found to have large eggs, 2 had small eggs, and the other 4 showed no ovarian development. In the same period adults obtained from larval diets failed to lay eggs even after 30 days of feeding.

Poor oviposition of College Station weevils was obtained on squares also. Whereas Mexican weevils had exceeded five eggs per female by the tenth day of feeding, the College Station weevils had not yet started to lay. In fact, they did not reach one egg per female per day until after 14 days. An examination of their ovaries after 21 days of feeding showed that 12 females were laying and 9 were not. These adults were the progeny of field-collected and square-reared weevils brought into the laboratory from September 12 to October 3. They were supplied adequately with squares, candles, blooms, and small bolls.

Overwintered weevils collected May 16, 1958, started laying after 3 weeks of feeding on a pollen diet. The last egg was laid on August 25 and all weevils had died by September 9. About 13 females laid 247 eggs, or 19 eggs per female (not corrected for mortality), most of them between June 21 and July 31.

Except for the tests with overwintered adults described above, most of the experiments were conducted with newly emerged unfed adults or those that had fed on other diets for no longer than 5 days. Oviposition diminished greatly when adults at the peak of egg laying were changed from squares to a pollen diet, although they began feeding on the artificial diet within 24 hours. A few eggs were laid, usually during the first 10 days.

Similarly, adults that were first conditioned to feed on an artificial diet ignored squares placed in the cages, sometimes for as long as 24 hours. They then gradually began to feed on the squares and, after several days no longer fed on the artificial diet. Females fed basal diet or other artificial diet for 5 days or more usually started depositing eggs the day after they were placed on squares.

Weevils reacted unfavorably also to a change from one artificial diet to another. However, they started feeding within 2 days if the new diet was attractive.

Since an increase in pollen concentration in the diets failed to bring egg production up to the level obtained for weevils fed on fresh squares, other ways of improving the adult diets were needed. Preliminary tests had shown that proteins and protein hydrolyzates were attractive to the adult weevils. Several of these substances were substituted for the enzymatic hydrolyzate of casein in an attempt to find a more attractive diet and one that would be better for egg production. Weevils were given a choice of two purified diets, each containing a different protein or protein hydrolyzate. Tests were run for 7 to 12 days, and feeding was observed each day. The adults were very sensitive to changes in the diets, and the amount of feeding was easily determined by examining the surface of the dish for frass and then removing the paraffin coating and observing the size of the holes.

Casein and wheat germ were more attractive than the protein hydrolyzates. Lactalbumin hydrolyzate, wheat hydrolyzate, and an amino acid mixture were slightly more attractive than the enzymatic hydrolyzate of casein. Protein hydrolyzates No. 2 and No. 7 and acid hydrolyzate of casein were similar to the enzymatic hydrolyzate of casein. Soybean protein was less attractive. Gelatin and two preparations of soy hydrolyzate were unattractive and adults fed very little.

Except for the wheat germ, the attractive substances tested caused no more oviposition in a 20-day period than the enzymatic hydrolyzate of casein.

On the basal diet A alone oviposition ranged from none to 75 eggs from 5 females in a 30-day period. Of these five only one was laying at the end of the test. Adults fed basal or crude diets survived well. The mortality rate on most diets compared favorably with the data shown in table 1.

During the course of the investigations with pollen, various changes were made in the basal diet to determine their effects upon oviposition. In one test all the constituents except the water and agar in basal B were doubled. No more eggs were obtained than from the usual concentrations of nutrients. Similarly, a twofold increase of nutrients in basal B and with 4 grams of pollen per 100 grams produced no increase in the number of eggs.

The substitution of an amino acid mixture for the enzymatic hydrolyzate of casein, and of a salt mixture (Vanderzant 1957) for Wesson's salts, and the addition of more vitamin mixture, inositol, α-tocopherol, carotene, lecithin, and nucleic acids did not affect oviposition.

There was no detectable reduction in oviposition when the pollen diets were heated. In fact, heating seemed to increase slightly the attractiveness of pollen, probably because part of the protein was broken down.

DIETS CONTAINING WHEAT GERM.—Although a few eggs were laid by weevils fed a number of crude substances, mainly plant materials, wheat germ was the only substance other than pollen or cotton-plant parts that promoted appreciable oviposition. In preliminary tests 2 grams of wheat germ per 100 grams of diet resulted in very little oviposition, but with 4 grams (table 3) good oviposition was obtained.

Table 3.—Oviposition by Mexican boll weevils on diets containing 4 grams of wheat germ per 100 grams.

MODIFICATION OF WHEAT GERM DIET	NUMBER OF FEMALES	NUMBER OF EGGS PER FEMALE IN—	
		20 Days	30 Days
None	12	1	—
		6	11
	10	11	21
	11	8	16
Enzymatic casein 1 gram and glucose 1 gram added	13	4	—
	9	4	—
Wheat germ 2 grams added	11	5	9
	9	9	18
Toasted wheat germ substituted	8	3	6
	10	0.6	1

Table 4.—Oviposition by laboratory-reared Mexican boll weevils on diets containing cotton-plant parts, 1958.

Cotton Part Added	Grams per 100 grams of Diet	Number of Females	Number of Eggs per Female in—	
			20 Days	30 Days
Cottonseed protein	1	12	6	13
	1	12	4	7
Hexane-extracted cottonseed meal	4	8	2	4
Cottonseed germinated 48–72 hours	10	6[a]	21	33
	10	5	5	—
	10	10	4	12
Newly emerged green seedlings	10	10	4	11
Leaves	2	6	2	—
	6	10	2	—
Squares	2	11	5	—
	6	10	6	14
Choice				
Squares and Germinated cottonseed	6		9	20
	10	11	2	5
Hexane-extracted cottonseed meal and Germinated cottonseed	4		2	3
	10	13	3	7
Germinated cottonseed and Green seedlings	10		6	—
	10	6	3	—
Green seedlings and Leaves	10		3	—
	6	10	0.3	—

[a] Square-reared.

In three of the four tests with the wheat-germ diet the number of eggs laid compared favorably with the number laid with pollen diet tests. When the enzymatic hydrolyzate of casein and glucose were increased, no improvement was noted, nor did an increase in the wheat germ improve egg laying. When toasted wheat germ was substituted for fresh wheat germ, oviposition was poor.

When fresh wheat germ was autoclaved with the other constituents of the adult diet, oviposition was greatly reduced, although the diet was still attractive to the adults.

Since the number of eggs per female varied markedly even with the same concentration of wheat germ, and the amount of heating was critical, wheat-germ diets were not used as substitutes for pollen diets.

DIETS CONTAINING COTTON-PLANT PARTS.—Several tests were conducted to determine the effect on oviposition of the addition to a basal diet of ether- or alcohol-extractable materials[6] from squares and blooms. Some extracts stimulated feeding, but no significant increase in oviposition occurred, presumably because some nutrient was lacking. However, it was reasonable to expect that oviposition would occur if an entire part of the cotton plant were added. After preliminary heating to stop enzyme action, homogenates of squares, blooms, leaves, germinated cottonseed, and seedlings were made and added to basal B diet. Also tested were cottonseed protein low in gossypol and cottonseed meal with the oil removed.

Mexican weevils fed readily on all diets except two containing the leaves, and on them feeding increased after the weevils became conditioned to them. When weevils were given a choice of diets containing cotton plant parts, the most attractive were those containing germinated cottonseed and squares. Green seedlings were almost as attractive; cottonseed meal and cottonseed protein were the least attractive.

The oviposition record of Mexican weevils on these diets is shown in table 4. Unfed adults from squares laid 33 eggs per female in 30 days on diets with germinated seed. With laboratory-reared adults, oviposition on diets containing germinated seed, seedlings, and squares was the same. Although less square material was added than germinated seed and seedlings, it appeared that feeding increased as the amount of square homogenate was increased; so higher concentrations were not tested. Similarly, leaf diets became less acceptable as the concentration was increased. In fact, oviposition on the leaf diets was not much better than on the basal alone.

The poor oviposition with cottonseed protein and cottonseed meal was probably caused, in part, by the poor feeding. However, the unusually good response obtained with germinated cottonseed indicated that additional nutrients were synthesized or made more available during the germination process. This result was in agreement with previous observations that the pink bollworm, *Pectinophora gossypiella* (Saund.), grew on germinated cottonseed, peas, and beans (Vanderzant *et al.* 1956) and that the boll weevil became sexually mature and oviposited on newly emerged green cotton seedlings (Vanderzant & Davich 1958). It has been observed recently that the boll weevil will oviposit on a basal diet containing germinated peas (*Pisum sativum*) with results similar to those for germinated cottonseed.

As an economical practical adult diet the germinated cottonseed diet appears to be promising. Since germinated cottonseed supplies many vitamins, minerals, proteins, amino acids, and lipides needed by the insect, some of the nutrients present in the basal diet probably can be omitted and the concentrations of others reduced when germinated seed is combined with it.

In several tests weevils were given a choice of two diets containing different cotton-plant parts (table 4). Squares and germinated cottonseed produced the same feeding response, but four times as many eggs were obtained on the diet containing squares. With cottonseed meal and germinated seed feeding was heavier, and twice as many eggs were laid on the latter diet. Feeding was the same on green seedling and germinated cottonseed diets, but twice as many eggs were obtained on the germinated seed. Green seedling diets were markedly preferred to leaf diets for both feeding and eggs. Feeding was the same on basal diets with and without gossypol.

As previously described, poor oviposition had been obtained on pollen diets with College Station weevils. However, it was expected that it would be better if weevils were given diets containing their natural food. Several groups of square-reared adults were given diets containing squares or blooms from September 15 to October 3 (table 5). Blooms gave better results than squares, but both failed to give as good oviposition as was expected. One group of weevils laid 10 eggs on the diet with squares during the first 15 days of the experiment. During the next 15 days these weevils were fed both the diet containing squares and the mixed pollen (2 grams per 100 grams) diet; during this period 40 eggs were laid. The weevils fed equally well on both diets, but laid more eggs on the diet containing squares.

[6] Obtained from Pesticide Chemicals Research Branch, Entomology Research Division, Beltsville, Maryland.

Table 5.—Oviposition by square-reared College Station boll weevils on artificial diets containing cotton plant parts, 1958.

Date Test Begun	Pretreatment	Cotton Part Added	Days in Test	Total Number of Eggs
September 15	Unfed	Squares	15[a]	10
16	Fed squares, 5 days	Squares	23	17
19	Unfed	Squares	25	17
		Blooms[b]	25	64
October 3	Fed seedlings, 5 days	Squares	25	0
		Blooms	28	2

[a] Given mixed pollen diet in addition for next 15 days—40 more eggs obtained.
[b] Eleven females used in this test; 14 in all other tests.

Since no eggs were laid by weevils started on cotton-containing diets in October, it was concluded that the weevils probably were in diapause. College Station weevils reared in the laboratory in October and November, even though they fed heavily, also failed to lay eggs in 30 days when fed several artificial diets that were successful for Mexican weevils. Tests were repeated several times with different adult diets, but no eggs were found.

DIETS THAT WERE REPELLENT.—Several substances used to fortify animal diets were tested (table 6). Unfortunately the nutritive value of these substances could not be determined because the weevils would not eat. In fact, when alfalfa meal and forage-juice concentrate were included, the adults starved to death because they would not go near the diets. Neither yeast extract nor soy hydrolyzate was attractive to them, and the combination was even less acceptable. These tests were repeated and similar results were obtained.

ADULT DIETS TESTED AS LARVAL DIETS.—The ultimate goal is to devise a diet that is satisfactory for both larva and adult, so that adults will deposit their eggs in the container used for rearing the larvae. Several adult diets were tested as larval diets and found satisfactory. Adults reared on a diet containing 2 grams of mixed pollen per 100 grams of basal B weighed an average of 10 mg. On the wheat-germ diet they averaged 9 mg. Addition of 2 grams of pollen or wheat germ or 10 grams of germinated cottonseed to the larval diet permitted better growth than on the larval diet alone. Adults from each of these diets weighed about 12 mg. as compared with 8 to 9 mg. for the soybean-protein larval diet. The greater concentrations of nutrients in these larval diets to which pollen, wheat germ, or seed was added undoubtedly account for the larger adults obtained. It seems likely that quality of nutrient is more important in adult nutrition than quantity, whereas both are important for the larvae whose growth demands a greater supply of nutrients.

Table 6.—Feeding response of adult boll weevils to basal diet A containing various additional substances.

Substance Added (Grams per 100 Grams)	Days in Test	Number of Weevils		Feeding Response
		Initial	Final	
Beef extract 2	12	9	6	Very light
Soy hydrolyzate 2[a] and yeast Extract 2.5	19	24	4	Very light
Distiller's dried solubles 3	8	19	1	Light
Alfalfa meal 2.5	10	25	1	None
Forage juice concentrate 2.5	7	21	0	None

[a] Substituted for enzymatic casein.

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Effect of Host Condition on the Clover Root Borer[1]

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ABSTRACT

Oviposition by the clover root borer, *Hylastinus obscurus* (Marsham), is completed earlier in weakened than in vigorous plants, but development is more rapid in vigorous or lightly infested roots. High populations occurring early in the season result in plant mortality with subsequently slowed borer development.

Schmitt (1844) and Riley (1879) believed that the clover root borer, *Hylastinus obscurus* (Marsham), preferred weakened plants for oviposition. Rockwood (1926) thought development was more rapid in weakened plants. Field observations made in Ohio in 1954 and 1955 showed that weakened plants had more and further-developed borers. However, reasons for these phenomena appear different from previously ascribed reasons.

During 1954 and 1955 separate samples of 30 roots each were taken of living and dead plants within the same field. In most cases root borer populations were considerably higher in the dead than in the living plants and borers were more advanced. However, from these observations alone it was impossible to conclude whether development was more rapid because of the weakened plant condition or if plants died because of early, heavy borer infestations. If plants die because of early, heavy infestations, one would expect the more heavily infested

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Table 1.—Root borer populations and development.

Date	Comparison	Stage of Development[a] Egg	Larva	Pupa	Adult	Total Borers	t	χ^2
		1954—Live vs. Dead plants						
July 26	Live		86	10	4	164	2.20*	20.5**
	Dead		66	22	12	245		
Aug. 2	Live		68	17	15	119	2.45*	27.7**
	Dead		38	27	35	200		
Aug. 9	Live		75	16	9	75	3.48**	57.1**
	Dead		27	16	57	177		
		1954—Living plants						
June 28	1–5	3	97			35		7.3*
	6–10	13	87			92		
	11+	0	100			34		
July 12	1–5	5	95	0		21		6.3*
	6–10	3	97	0		117		
	11+	10	89	1		157		
July 26	1–5	3	93	0	3	29		14.2**
	6–10	1	88	8	3	74		
	11+	2	77	17	4	64		
		1954—Dead plants						
July 26	1–5		79	13	8	24		5.2
	6–10		60	25	15	106		
	11+		69	22	9	115		
Aug. 2	1–5		43	30	27	54		8.3*
	6–10		25	25	50	59		
	11+		43	27	30	86		
Aug. 9	1–5		44	16	40	45		13.9**
	6–10		14	18	68	71		
	11+		30	13	57	61		
		1954—Old vs. New field						
May 31	Old	20	80			51		22.1**
	New	65	35			52		
June 7	Old	16	82	2[b]		51		8.1**
	New	41	59	0		54		
June 20	Old	0	100			101		22.0**
	New	19	81			108		
June 28	Old	0	96	4		50		7.9**
	New	14	86	0		120		
		1955—Early vs. Normal cutting						
July 5	Early	6	90	4		138	2.94**	20.0**
	Normal	27	73	0		71		
July 12	Early	3	83	13	1	179	1.29	10.3**
	Normal	8	88	4	0	113		
July 19	Early	5	70	16	9	148	1.83	15.5**
	Normal	8	84	8	0	112		
July 26	Early	3	71	14	12	152	1.24	14.9**
	Normal	9	81	7	3	112		
Aug. 2	Early	1	58	27	14	85	0.46	19.3**
	Normal	8	75	14	3	118		
		1955—Normal cutting						
June 20	1–4	36	64			39		10.5**
	5+	10	90			69		
July 5	1–4	50	50			32		15.9**
	5+	8	92			39		
Aug. 9	1–4	0	50	36	14	36		11.1**
	5+	3	72	13	12	143		
Aug. 16	1–4	3	49	23	25	39		9.7**
	5+	0	75	16	9	131		
		1955—Early cutting						
July 5	1–4	21	68	11		28		17.6**
	5+	2	96	2		120		
July 12	1–4	4	81	15		26		0.6
	5+	3	84	13		152		
July 19	1–4	3	59	16	22	32		9.0*
	5+	6	73	16	5	116		

[a] Per cent of total borers.
[b] Probably from overwintering larvae.
* Significant at 5% level; ** at 1%.

plants to contain borers more advanced in their development. To check this theory, samples taken in 1954 were also divided into three categories; roots with 1 to 5, with 6 to 10, and with 11 or more borers. Roots taken in 1955 samples were separated into two categories, those containing one to four borers and those harboring five or more. Because of the great number of samples involved, only those taken during the periods in which significant differences occurred are summarized in table 1. Samples taken after August 20 in no case showed differences in stages of borers present but this condition was likely caused by the fact that most borers became adult at this time and their age could not be reliably determined.

Chi-square was used to compare borer development under different plant conditions. Chi-square values were calculated from the actual number of borers in each stage—egg, larva, pupa, or adult—but these data are expressed as percentages in table 1 for ease of interpretation. No attempt was made to separate larvae by instar. If less than four borers were present in any stage, these were lumped with the next stage for analysis. To compare total number of borers present, individual root counts were transformed by the inverse hyperbolic sine (Pruess & Weaver 1959) and the t test was used to test for differences in transformed totals.

1954 SAMPLES.—In the field sampled in 1954, dead plants on all dates contained more and farther advanced borers than living plants, as shown in table 1. In living plants, differences in stage of development were significant on three dates. These samples were all taken early in the season. Borers in the heavily infested roots were generally more advanced, presumably because they had an earlier start. However, turning to samples of dead roots, we find that in general those plants having 6 to 10 borers had the most advanced borers and those with 1 to 5 the most delayed. The very heavily infested plants (11 or more borers), though presumably infested earlier than those with less infestation, probably had development somewhat delayed, owing to competition, while the lightly infested plants were the last ones infested and therefore had younger borers. Further evidence for such a situation will be seen in the 1955 samples.

1955 SAMPLES.—A few plants in the field sampled in 1954 lived over into 1955. Oviposition began about a week sooner in these roots than those in a nearby new field. These old plants were severely weakened and most of them had died by June 1. However, when sampling was concluded on June 28 larvae were almost 2 weeks farther advanced than in the new field, as evidenced by size of larvae and appearance of first pupae. The author (Pruess 1957) previously concluded that development was more rapid because of the weakened condition of the plants. However, reexamination of the data showed that all oviposition in the old field had ceased by June 20 while many eggs were still being laid in the new field at this time. This continued increase in population would, of course, keep the average age of borers younger.

Part of the new field sampled in 1955 was clipped on May 11 just after spring migration ended. Repeated clipping so weakened this area of the field that most plants were dead by the middle of June. Differences in total numbers of borers in the two parts of this field were significant only in the first sample taken on July 5. Numbers

tended to equalize as the season advanced. It seems that this difference was due largely to the earlier completion of oviposition and hatching of larvae in the weakened plants. Although the number of adult root borers was almost identical in the two areas, those ovipositing in vigorous plants were laying more eggs in July, while the higher populations in the weakened plants at this time indicated that many eggs were laid sooner in this part of the field. The low population observed on August 2 in the weakened plants was probably not representative. At this time heavily infested plants in the early cut portion were so badly decayed that many borers were undoubtedly missed despite attempts to choose plants at random, regardless of condition. Even more outstanding than the difference in population was the stage of development of borers in the two parts of the field. Chi-square tests showed that borers were much more advanced in the early-cut portion. However, this again may be attributed largely to the fact that oviposition was completed sooner with subsequent earlier hatching and maturation of larvae in this section of the field.

In the weakened early-cut portion of the field it was found that on July 5 the heavily infested plants had the farthest advanced borers, no difference on July 12, and that by July 19 the trend had already reversed. After this period, however, no difference was evident, probably because all plants, regardless of root borer infestations, had become badly decayed and furnished a poor substrate for borer development.

In the normally cut portion of the field it was noted that those plants having the most borers also contained the more advanced borers early in the season, but that later the reverse was again true. As one would scarcely expect host condition to greatly affect the time required for hatching of eggs, we can conclude only that the reason for a higher percentage of pupae and adults in the heavily infested roots was that these eggs were laid sooner. It also appears that borer growth was slower in the heavily infested roots, as judged by the delayed development in these roots in the samples taken August 9 and 16.

It must be realized that the conclusions drawn here were based on random samples taken under uncontrolled field conditions. Further laboratory and field studies would be desirable. However, the type of tests used leaves no doubt that real differences did exist and the conclusions set forth herein seem to explain these differences satisfactorily.

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Effect of Pre-seeding Insecticide Treatments of the Seed Bed on Red Clover Stands and Forage Yields[1]

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ABSTRACT

Massive dosages of five insecticides, DDT, BHC, chlordane, toxaphene, and parathion, and a fungicide, Arasan, were applied to soil and disked in with a rotary hoe. Kenland, Midland, Wisconsin Mildew Resistant, and Michigan Common varieties of medium red clover were planted in the insecticide-treated soils. The best agronomic, cultural, and insect- and disease-control practices were followed. Insect populations of the crop year on July 11, 1951, showed marked differences among the treatments, particularly for grasshoppers, aphids, Diptera, and Hymenoptera. Differences that were highly correlated with data taken the following year occurred in stand among the various treatments in the seeding year. The effect of BHC in reducing the stand of grasses and the companion oat crop was marked. Highly significant differences in stand were present in the spring of the crop year. Yields when corrected for stand differences were significantly affected. Second-crop forage yields showed notable differences among the varieties, with Kenland outstandingly good. The BHC plots produced the highest yields. The final stands on October 17, 1951, showed that plots treated with BHC or with a mixture of insecticides maintained the stand best for all varieties, but were particularly effective for Wisconsin Mildew Resistant and Midland. Kenland went into the second winter with an 84% stand.

Failure of red clover stands after the first crop year is a common occurrence throughout the Midwest. Since red clover plants can be maintained for several years under greenhouse culture, it seems apparent that crop failure in the field is probably caused by factors not present under greenhouse conditions. This experiment attempted to maintain stands of red clover by applying the latest information on cultural practices and insect and disease control. However, owing to conditions beyond the control of the investigators, the experiment had to be abandoned at the end of the first crop year.

Little has been done with modern fungicides to control diseases of red clover in the field. The advent of DDT and other organic insecticides resulted in excellent control of insects both in the soil and those feeding on the foliage and floral parts. Marshall *et al.* (1949) reported control of the clover root borer, *Hylastinus obscurus* (Marsh.), and other clover insects with spring applications of insecti-

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cides to the plants. App & Everly (1949) obtained similar results with applications of insecticidal dusts in the fall of the seeding year and in the spring of the crop year. Studies on the cultural treatment of the companion crop residues in newly seeded clover fields by Willard & Lewis (1947) in Ohio indicated that the presence of excessive amounts of straw or crop growth in the fall reduced the stands of clover.

EXPERIMENTAL PROCEDURE.—On May 10 and 11, 1950, insecticidal dusts were applied with a 3-foot Gandy spreader to plot areas prior to seeding with red clover. One-half the amount of dust was applied one way of the plots and the remainder in strips at right angles to the first application. The dusts were worked into the top 6 inches of the soil with a rotary hoe. After the clover was seeded, the whole area was planted to oats as a companion crop.

The experimental design consisted of three replicates with the insecticide soil treatments as randomized main plots. The four varieties of red clover were randomized as subplots of the main insecticide plots.

The varieties of clover used were Kenland, Midland, Wisconsin Mildew Resistant, and Michigan Common and were seeded at the rate of 12 pounds per acre. The insecticide dusts used and the amounts of active ingredient in pounds per acre were: 2% DDT—100; ½% BHC—15 gamma isomer; 1% chlordane—50; 2% toxaphene—100; ½% parathion—15; Arasan®(bis(dimethylthiocarbamoyl)disulfide)-8; and a mixture of the above dusts containing DDT—30, BHC—5 gamma isomer, chlordane—15, toxaphene—30, and parathion—5; All the insecticide dusts included 8 pounds of active ingredient of Arasan per acre.

To reduce chances of reseeding during the experiment, the clover seed was scarified before planting to reduce the numbers of new plants developing later from hard seed that failed to germinate promptly. In addition, the plots were clipped in early September before any flowers had set seed and the hay and straw stubble were removed. During the crop year, after the samples to determine forage yields were taken, the entire area was mowed and the hay removed.

Foliage sprays containing DDT, dieldrin, and BHC at 2, 1, and ½ pounds of active ingredient per acre, respectively, were applied to the clover foliage on July 11 and September 11, 1951. On May 1, 1951, BHC at the rate of 1.9 pounds of gamma isomer per acre was applied to all plots receiving soil treatments as a ½% dust with a Gandy spreader.

Insect populations were sampled on July 11, 1951, prior to the insecticide spray application by taking 10 sweeps in each main insecticide plot across the varieties so that all varieties were equally represented in the sampling.

On July 11, 1950, the number of clover plants per square foot was determined by taking three samples of 1 square foot each in each subplot. As part of this, the numbers of other plants present were counted. On April 30, 1951, a visual rating of each subplot for stand and vigor was made by two independent observers. Immediately following these ratings, stand indices were obtained by using a grid of 25 6″×6″ squares. These grids were placed in the plots at random and the squares containing no clover plants were counted. Two samples were taken in each subplot. On October 17, 1951, this operation was repeated to obtain stand indices of the clover in the plots prior to the second winter.

Forage yields were taken on June 12 and August 14, 1951. An area of 54 square feet in each subplot was harvested and weighed. On August 14 a visual rating of the amount of weed growth in each plot was made by two independent observers.

REVIEW OF LITERATURE.—A review of previous studies of the effects of massive dosages of soil additives on legume crops and other plants present in the fields will aid in properly interpreting the data from these experiments. Appleman & Sears (1946) reported growth of red clover, sweet clover, and lespedeza retarded by dosages of 1,000 pounds of active ingredient of DDT per acre. They also found that at a rate of 10 pounds of DDT per acre nodules formed only on the tap root; at 100 pounds, nodules formed on roots only below the treated area. At a 1,000-pound rate the nodules formed were very small and roots were severely injured. All plants showed shorter growth at the two higher dosages. Injury to plants and soil organisms varied with the type of culture media. Dosages of DDT from 0 to 1,000 pounds per acre caused less stunting of growth and reduction of nodules in soil than in sand. Wilson & Choudhori (1948) stated that BHC at 30 p.p.m. in soil severely injured red clover, soybeans, alfalfa, and hairy vetch. At 3 p.p.m. some injury was visible. Plants made little growth at rates of 500 and 1,000 p.p.m. They found that nitrogen-fixing bacteria were not injured. Some differential effects with other species of soil organisms were noted. Simkover & Shenefelt (1951) reported that BHC in the soil reduced damping off of Norway pine seedlings by a virulent strain of *Rhizoctonia*. Chlordane at 10 pounds and BHC at 1 pound gamma had no effect on nodules of nitrogen-fixing bacteria on roots of black locust. Kulash (1947) found that BHC injured sweet corn when the chemical was added to the soil either as a dust or spray. DDT caused no injury. Morrison *et al.* (1948) reported that DDT, chlordane, and toxaphene at dosages of 106.8, 27.5 and 27.5 pounds of active ingredient per acre, respectively, caused some stunting and chlorosis of lima beans. When DDT was increased to 137.5 pounds severe stunting of bush and pole beans and slight stunting of tomato transplants occurred, resulting in small leaves and fruits. BHC at 10 pounds of gamma isomer per acre caused no injury to beans. At 27.5 pounds, BHC caused varying degrees of injury to different varieties of legumes and beans. They found that BHC controlled lambsquarters, pigweed, and bindweed for more than 5 months. Little effect on grasses was evident, but common groundsel produced no seed for 5 months. Treatments resulted in off-flavor in potatoes and sweet corn. Foster (1951) found various plant responses when insecticides were added to the soil. In 1955, following Foster's work, Boswell *et al.* (1955) reported extensive studies in various parts of the United States with some controversial results, which were probably associated with soil types. They found DDT at 119 and 238 pounds active ingredient per acre severely stunted Black Valentine beans by shortening the internodes. Yield of pods was also reduced. Results from similar tests in 1951 resulted in severe reductions in yields. Lima beans

Table 1.—Red clover plants per square foot in plots 2 months after soil was treated with insecticides and fungicides prior to seeding. Lafayette, Indiana, July 11, 1950.[a]

VARIETY	DDT	BHC	CHLOR-DANE	TOXA-PHENE	PARA-THION	MIXTURE	ARASAN	UN-TREATED	VARIETY AVERAGE
Kenland	3.9	17.8	15.5	12.6	12.0	14.5	11.2	12.0	12.4
Midland	4.9	16.8	12.3	11.5	7.6	15.9	11.3	12.5	11.6
Wis. Mildew Resistant	7.6	16.1	15.1	11.9	6.4	10.7	9.2	12.2	11.6
Mich. Common	6.4	13.6	9.3	12.9	8.6	9.8	13.9	7.2	10.2
Insecticide average	5.7	16.0	13.0	12.2	8.6	12.7	11.4	11.0	11.4

[a] All differences are nonsignificant. Data based on the average of three samples of 1 square foot in each subplot.

were much less injured and Red Mexican field beans suffered no injury. BHC at 15 pounds gamma per acre reduced stands of Black Valentine beans 75% and had less effect on Red Mexican beans. Probst & Everly (1957) found DDT at 50 and 100 pounds per acre reduced stands of soybeans significantly. BHC at 15 and 20 pounds gamma per acre delayed germination and reduced stands, but only the higher dosage affected yields. Other insecticides tested that showed no effects were EPN, chlordane, methoxychlor, heptachlor, dieldrin, aldrin, and toxaphene.

It is apparent that extremely heavy dosages of some insecticides affect legumes adversely, either through direct action on the plants or on the soil microorganisms associated with their growth. Soil types also contributed materially in modifying these effects.

CLOVER AND WEED STANDS IN 1950 COMPARED WITH THOSE IN 1951.—Very little information was obtained in 1950, since it was the seeding year. The principal objective was to provide conditions for good growth and stand maintenance by the use of the best cultural practices with respect to companion crop harvest and disposition of the crop residue.

After the oats had been removed, stand counts of clover plants and extraneous plants were made. The data on the clover stand are given in table 1. Although there were wide differences in the stand among the various soil treatments, the large amount of variability in the experiment made these differences nonsignificant. The best stands at this time were in the BHC soil treatment and the poorest in the DDT. There was very little difference between the varieties, although Kenland had the most plants per square foot and Michigan Common the least.

Even though these differences were considered nonsignificant, subsequent data taken during 1951 showed the influence of these early differences. To test the degree of influence this early stand had on subsequent data, correlations between the original stand on July 11, 1950, and all the agronomic data taken later were made. These correlations for the soil treatments as well as for varieties are given in table 2.

The original differences in stand among the varieties were significantly correlated with only the visual ratings and stand indices taken April 30, 1951, although the correlation with weed indices on August 14, 1951, was nearly significant. Since the original stand for the varieties and among soil treatments shows notable correlations with all but two of the collections of data taken in 1951, it appears that these original stands contributed importantly to subsequent performance of clover plants in the test. The two sets of data showing no significant correlation with the original stands were the green weight of forage yields on June 12 and August 14, 1951. When these yields were corrected for the amount of weeds present, significant correlations are shown. Thus, differences in the numbers of clover plants on July 11, 1950, among the various soil treatments, although nonsignificant within the data from which they were derived, take on importance from their effect on subsequent information from these plots and therefore are believed to be real differences.

The data on the numbers of plants per square foot other than red clover are given in table 3. It is apparent that significantly less weeds occurred in the BHC and the mixed insecticide plots containing BHC than in the other treatments. This was largely caused by the elimination of all grasses, including the companion crop. Although these extraneous plants represented only broadleafed plants, these same treatments also contained the fewest weeds in October 1951. There were no consequential differences in the number of weeds present among the four varieties of red clover. Michigan Common was the only variety that showed an important reduction in the numbers of weeds in any treatment other than the BHC and mixed insecticides. Highly significantly fewer weeds were present in this variety in the parathion treatment than in the untreated.

STAND AND VIGOR IN 1951.—Visual ratings for stand and vigor of the clover in the experiment were made on April 30, 1951. Observations were made by two independent observers and evaluations were averaged and are presented in table 4. At this time outstanding differ-

Table 2.—Correlations between the original stand on July 11, 1950, in red clover plots with soil treated with insecticides and fungicides prior to seeding, and subsequent data taken from these plots in 1951. Lafayette, Indiana.

TABLE NO.	STAND COUNTS ON JULY 11, 1950 CORRELATED WITH:	FOR SOIL TREATMENTS	FOR VARIETIES
4	Visual ratings, April 30, 1951	0.917**	0.970*
5	Stand indices, April 30, 1951	.888**	.979*
6	Weight of forage, June 12, 1951	.682	.095
7	Weight of forage corrected for stand differences, June 12, 1951	.810*	.858
9	Weed indices, August 14, 1951	.879**[a]	.946[a]
10	Weight of forage August 14, 1951	.250[a]	.620
11	Weight of forage corrected for weeds, August 14, 1951	.813*	.829
12	Weight of forage, August 14, 1951 corrected for stand, Oct. 17, 1951	.708*	.738
13	Stand indices, October 17, 1958	.795*	.735
14	Ratio of forage, August 14, 1951 to that on June 12, 1951	.667	.809
	r values required for significance 19:1	.707	.950
	99:1	.834	.990

[a] Negative correlations, all others positive.
** Differences significant at 1% level.
* Differences significant at 5% level.

Table 3.—Plants other than red clover per square foot in plots 2 months after soil was treated with insecticides and fungicides prior to seeding. Lafayette, Indiana. July 11, 1950.[a]

Variety	DDT	BHC	Chlordane	Toxaphene	Parathion	Mixture	Arasan	Untreated	Variety Average
Kenland	75.0	30.3	69.7	57.3	62.3	37.7	76.3	82.3	61.8
Midland	67.3	40.0	60.7	53.0	56.0	49.3	70.3	79.0	59.4
Wis. Mildew Resistant	63.0	24.3	60.0	51.0	59.3	43.3	69.3	79.0	56.0
Mich. Common	80.0	29.0	64.0	56.3	38.7	47.7	85.3	77.3	60.2
Insecticide average	71.3	31.8	63.6	54.4	54.9	44.5	75.1	79.4	59.4

L.S.D. for odds:	Insecticides	Varieties	Varieties for given insecticide	Insecticides for given variety
19:1	23.2	n.s.	n.s.	23.8
99:1	32.2	n.s.	n.s.	31.3

[a] Data are averages of 3 samples of 1 square foot each from each subplot.

ences among the treatments were apparent. These visible differences were substantiated by the stand indices count made at the same time as shown on table 5. Highly significant positive correlation coefficients for soil treatments and varietal means between the visual ratings and the stand indices counts taken the same day were 0.904 and 0.999, respectively. This high correlation value indicates that visual ratings for stand and vigor were as accurate as those measured by the sampling grid method.

At the beginning of the crop year, the Kenland variety had the best stand and vigor, while Midland and Wisconsin Mildew Resistant were better than Michigan Common. Among the insecticide treatments the BHC plots had the best stand with the mixed insecticides, and with toxaphene next in order. The DDT plots had the poorest stand. The stand and vigor of the chlordane, parathion, Arasan, and the untreated plots were very similar.

First Crop Yield of Forage in 1951.—Sample areas of 54 square feet were harvested for forage yields from each subplot on June 12, 1951. The weights of these samples are given in table 6. There were no meaningful differences in yields among the varieties, although Wisconsin Mildew Resistant had the highest and Michigan Common the lowest yield. The differences in yield among the various soil treatments were caused mainly by the low yield of the DDT-treated plots. With the exception of the DDT-treated plots, all the other treatments had higher yields than the untreated. Since these forage samples consisted of mixtures of red clover and weeds, it is questionable whether weeds would yield as much forage as the clover plants. The presence of weeds in greater amounts in certain plots, such as the DDT, would tend to mask some of the real differences in the yield of the clover.

To measure the amount of red clover forage in the sample yields given in table 6, the index of stand taken on April 30 was used to adjust these yields for the varying numbers of clover plants in the different plots. These corrected yield data are given in table 7. Using these corrected yields as more nearly representing the actual yield of clover hay, the variety Kenland had the greatest yield, although only slightly better than Wisconsin Mildew Resistant. As before, Michigan Common had the lowest yield. Among the soil treatments, the DDT plots showed only about half as much yield, indicating that weeds comprised a great portion of the original yield. The other treatments showed reductions in varying amounts, the least in the chlordane and mixture-treated plots and the greatest in the untreated. Although the soil treatments which had yields significantly greater than the untreated showed no change, the corrected data give a truer picture of the actual effects of the soil treatments on the yield of red clover.

Insect Populations in 1951.—Only one collection of insects was made just prior to a foliage application of insecticides on July 11, 1951. Collections were made by soil treatment blocks, but all varieties in each block were included proportionally in the collections. The numbers of the more important insects found in the different soil treatments are given in table 8. Grasshoppers were exceedingly abundant, averaging 13 per sweep in the untreated plots. The plots treated with BHC, chlordane, and the mixture of insecticides had the fewest. However, these differences were nonsignificant, owing to the great amount of variation in the data. The plots in which the soil was treated with toxaphene, which gives good grass-

Table 4.—Visual ratings for stand and vigor of red clover in plots 12 months after soil was treated with insecticides and fungicides prior to seeding. Lafayette, Indiana, April 30, 1951.[a]

Variety	DDT	BHC	Chlordane	Toxaphene	Parathion	Mixture	Arasan	Untreated	Variety Average
Kenland	1.3	9.0	5.7	6.3	5.0	7.3	5.3	5.7	5.7
Midland	1.0	8.0	4.7	6.7	5.0	6.3	4.3	5.3	5.2
Wis. Mildew Resistant	1.7	7.7	5.3	4.7	4.3	7.3	4.3	5.3	5.1
Mich. Common.	1.0	7.3	3.3	4.7	4.0	5.3	3.7	2.7	4.0
Insecticide average	1.2	8.0	4.7	5.6	4.6	6.6	4.4	4.7	5.0

L.S.D.	Insecticides	Varieties	Varieties for given insecticide	Insecticices for given variety
19:1	2.1	0.5	1.4	2.0
99:1	2.9	1.2	1.9	2.6

[a] Index of 1 is poor stand and vigor, to 9 excellent stand and vigor.

Table 5.—Stand indices of red clover in plots 12 months after soil was treated with insecticides and fungicides prior to seeding. Lafayette, Indiana, April 30, 1951.[a]

VARIETY	DDT	BHC	CHLORDANE	TOXAPHENE	PARATHION	MIXTURE	ARASAN	UNTREATED	VARIETY AVERAGE
Kenland	11.3	24.3	22.0	23.8	23.2	23.6	22.7	21.7	21.6
Midland	10.3	24.3	22.0	22.8	19.7	23.0	20.7	22.0	20.6
Wis. Mildew Resistant	10.0	24.7	23.0	20.2	20.0	22.3	21.5	20.8	20.3
Mich. Common	8.3	23.2	22.2	21.0	19.0	21.0	18.8	14.0	18.4
Insecticide Average	10.2	24.1	22.3	22.0	20.4	22.4	20.9	19.6	20.2

L.S.D.	Insecticides	Varieties	Varieties for given insecticide	Insecticides for given variety
19:1	3.4	1.6	4.4	4.6
99:1	4.8	2.1	5.9	6.2

[a] Number of 6-inch squares out of 25 having clover plants or foliage. Multiplying by the factor four will convert these data to per cent stand.

Table 6.—Green weight of red clover forage harvested from plots 13 months after soil was treated with insecticides and fungicides prior to seeding. Lafayette, Indiana, June 12, 1951.[a]

VARIETY	DDT	BHC	CHLORDANE	TOXAPHENE	PARATHION	MIXTURE	ARASAN	UNTREATED	VARIETY AVERAGE
Kenland	16.3	24.1	24.4	23.6	25.9	28.3	22.6	21.0	23.3
Midland	13.2	24.8	26.7	24.8	23.6	27.0	21.8	24.5	23.3
Wis. Mildew Resistant	17.3	25.2	26.8	24.7	27.1	25.4	26.1	23.3	24.5
Mich. Common	7.4	22.6	25.2	24.4	27.6	26.0	24.7	21.5	22.4
Insecticide average	13.6	24.2	25.8	24.4	26.0	26.7	23.8	22.6	23.4

L.S.D.	Insecticides	Varieties	Varieties for given insecticides	Insecticides for given variety
19:1	4.5	n.s.	n.s.	4.4
99:1	6.3	n.s.	n.s.	6.0

[a] Average yield in pounds from 54 square feet harvested from each subplot.

Table 7.—Green weight of red clover forage, corrected for weeds present, and harvested from plots 13 months after soil was treated with insecticides and fungicides prior to seeding. Lafayette, Indiana, June 12, 1951.[a]

VARIETY	DDT	BHC	CHLORDANE	TOXAPHENE	PARATHION	MIXTURE	ARASAN	UNTREATED	VARIETY AVERAGE
Kenland	7.5	23.5	21.8	22.5	24.0	27.1	20.5	18.2	20.6
Midland	6.2	24.1	23.6	22.7	19.6	24.9	18.0	21.4	20.1
Wis. Mildew Resistant	8.0	24.9	24.4	20.0	21.6	22.8	22.4	19.4	20.4
Mich. Common	2.8	20.9	22.6	20.4	21.5	21.5	18.9	12.6	17.6
Insecticide average	6.1	23.4	23.1	21.4	21.7	24.0	20.0	18.0	19.7

L.S.D.	Insecticides	Varieties	Varieties for given insecticide	Insecticides for given variety
19:1	6.4	n.s.	n.s.	8.5
99:1	8.8	n.s.	n.s.	11.6

[a] Yield data in pounds from table 6 corrected for differences in stand of clover plants as shown in table 5, using the regression method.

Table 8.—Number of insects collected from red clover plots 14 months after soil was treated with insecticides and fungicides prior to seeding, Lafayette, Indiana, July 11, 1951.[a]

INSECTICIDE	GRASSHOPPERS	PLANT BUGS[b]	APHIDS	LEAFHOPPERS[c]	ADULT SPITTLEBUGS	*Orius insidiosus* (Say)	DIPTERA	COLEOPTERA	HYMENOPTERA	LEPIDOPTERA
DDT	16.2	2.2	2.6*	5.0	4.0	2.0	22.3	1.5	1.4*	0.1
BHC	5.7	1.4	0.8*	4.8	1.7	1.8	14.9**	0.8	0.9*	0.0
Chlordane	5.4	1.5	3.9	4.8	2.6	2.1	14.2**	1.0	1.0*	0.1
Toxaphene	8.6	1.8	4.2	6.5	3.6	2.6	20.3*	1.1	1.6*	0.1
Parathion	10.5	1.5	3.2	5.5	3.0	2.7	21.2	1.3	1.1*	0.0
Mixture	4.8	2.1	3.2	5.5	2.3	1.7	17.8**	1.1	2.8	0.1
Arasan	12.8	2.0	4.1	4.7	3.4	2.9	25.6	1.4	2.6	0.
Untreated	13.0	1.5	5.1	3.2	3.4	2.2	24.2	1.2	2.9	0.2
L.S.D.										
19:1	n.s.	n.s.	2.2	n.s.	n.s.	n.s.	3.9	n.s.	1.1	n.s.
99:1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	5.4	n.s.	n.s.	n.s.

* Differs a significant amount from the untreated.
** Differs a highly significant amount from the untreated.
[a] Average per sweep based on 10 sweeps in each main insecticide plot.
[b] Includes *Lygus lineolaris* (P.deB.), *Adelphocoris rapidus* (Say), and *A. lineolatus* (Goeze).
[c] Includes numerous species, but *Empoasca* sp. and the clover leafhopper *Aceratagallia sanguinolenta* (Prov.) predominated.

Table 9.—Visual ratings for weeds present in red clover plots 15 months after soil was treated with insecticides and fungicides prior to seeding. Lafayette, Indiana, August 14, 1951.[a]

Variety	DDT	BHC	Chlordane	Toxaphene	Parathion	Mixture	Arasan	Untreated	Variety Average
Kenland	6.7	1.1	2.3	2.0	2.0	1.7	2.3	2.7	2.6
Midland	7.3	2.7	4.3	3.3	5.0	3.0	4.0	4.3	4.2
Wis. Mildew Resistant	7.7	2.3	5.0	4.0	5.7	2.7	4.7	6.3	4.8
Mich. Common	9.0	3.0	5.3	3.7	4.3	4.0	5.7	7.0	5.2
Insecticide average	7.7	2.3	4.2	3.2	4.2	2.8	4.2	5.1	4.2

L.S.D. for odds of:	Insecticides	Varieties	Varieties for given insecticide	Insecticides for given variety
19:1	2.0	0.6	1.6	2.0
99:1	2.8	0.7	2.1	2.7

[a] Ratings based on 1—few or no weeds to 9, mostly weeds with little clover.

hopper control as a foliage spray or dust, had relatively few grasshoppers. However, parathion, also an effective grasshopper insecticide, had almost as many grasshoppers as the untreated. This condition may be explained by the fact that parathion breaks down rather rapidly under field conditions. The numbers of aphids per sweep were very low in the BHC plots and differed significantly from the untreated as also did the DDT-treated plots. As foliar applications, both these chemicals gave good control of the pea aphid, *Macrosiphum pisi* (Harr.). The numbers of Diptera collected in the various plots differed significantly. The toxaphene-treated plots showed considerably less and the BHC, chlordane, and the mixed insecticide plots importantly fewer flies than the untreated. All the treated areas showed greater numbers of leafhoppers than the untreated, although the variability of the data indicated these were nonsignificant. Meadow spittlebugs were almost eliminated from all plots by the early application of BHC. The adults represented in the collections were migrants from outside areas. No significant differences occurred in the numbers present, although the fewest were taken in the BHC-treated plots. Coleoptera were too few to be of any importance. Hymenoptera taken in the different treatments were significantly fewer in the DDT, BHC, chlordane, toxaphene, and parathion plots than in the untreated, with the fewest occurring in the BHC plots. In general the BHC-treated plots showed the fewest insects.

Weed Estimates in 1951.—On August 14, 1951, prior to the second-crop hay harvest, the plots were visually evaluated for the quantity of weeds present. These visual estimates are given in table 9. Differences, both among the varieties and the soil treatments were highly significant. In general the differences followed a somewhat similar pattern as for the extraneous plant counts made July 11, 1950, with the BHC-treated plots and the mixed insecticide plots having the fewest weeds.

Kenland had considerably less weeds than any of the other varieties. Midland and Wisconsin Mildew Resistant did not differ significantly from each other but both had notably fewer weeds than Michigan Common. This general pattern was shown in most of the insecticide-treated plots.

Among the insecticide-treated plots, DDT had the most weeds present, being much more weedy than any of the other treatments, including the untreated. Only the BHC plot had notably fewer weeds than the untreated, although the mixture of insecticide treated plots had significantly fewer.

Second Crop Hay Yield in 1951.—Samples to determine the amount of forage produced by the second growth of the clover were taken August 14, 1951. Areas of 54 square feet, the same as those used in the first crop yield samples, were cut and weighed and the pounds of forage harvested from each treatment are shown in table 10. There were no great differences among the yields of the soil-treated plots, although all had higher yields than the untreated. There were, however, highly important differences in the yields of the four varieties. The yield from Kenland was much greater than any of the other varieties. Midland and Michigan Common did not differ greatly from each other but both produced considerably greater yields than Wisconsin Mildew Resistant.

To obtain a better measure of the actual differences in the yield of the red clover in each plot, the data on gross yields shown in table 10 were corrected, using the esti-

Table 10.—Green weight of red clover forage harvested from plots 15 months after soil was treated with insecticides and fungicides prior to seeding. Lafayette, Indiana, August 14, 1951.[a]

Variety	DDT	BHC	Chlordane	Toxaphene	Parathion	Mixture	Arasan	Untreated	Variety Average
Kenland	12.6	13.4	11.0	12.6	11.3	13.6	10.6	10.1	11.9
Midland	11.5	9.8	8.0	8.9	9.7	9.9	8.9	6.8	9.2
Wis. Mildew Resistant	9.0	8.2	8.1	7.6	8.4	8.4	7.7	7.3	8.1
Mich. Common	11.1	10.1	8.3	10.2	9.7	8.4	10.1	8.6	9.6
Insecticide average	11.0	10.4	8.8	9.8	9.8	10.1	9.4	8.2	9.7

L.S.D. for odds of:	Insecticides	Varieties	Varieties for given insecticide	Insecticides for given variety
19:1	n.s.	0.6	1.8	n.s.
99:1	n.s.	0.7	2.4	n.s.

[a] Average weight in pounds of 54 square feet harvested in each subplot.

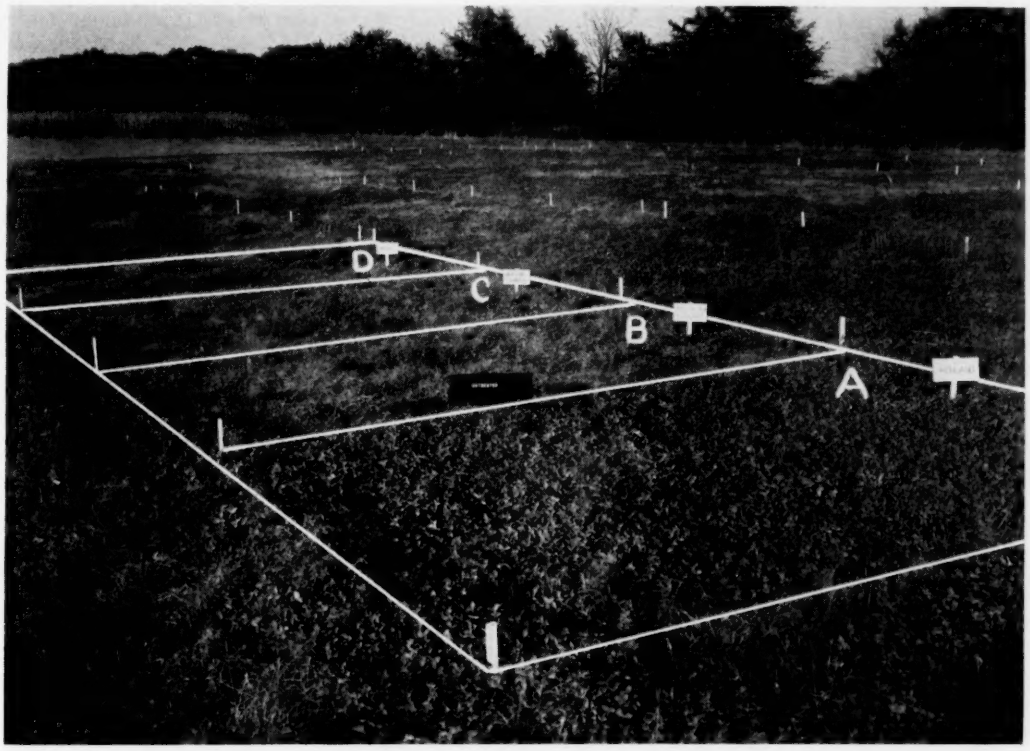

FIG. 1.—Residual stand of red clover on October 18, 1957, in untreated soil at the end of first crop year. A—Kenland, B—Wisconsin Mildew Resistant, C—Michigan Common, D—Midland.

mates of weeds given in table 9. These corrected data are given in table 11. The removal of the influence of weeds in the plots did not change the ratings of the varieties, although the yields were reduced. Kenland was still the outstandingly best producer and Wisconsin Mildew Resistant the poorest. The greatest effect of weeds was shown in the yields of the insecticide soil treatments. The yield from the BHC plots was greater than that for the chlordane and Arasan plots and much greater than DDT and the untreated plots. In addition, the mixed insecticides and the toxaphene-treated plots gave highly significantly greater yields than the untreated.

In order to study the effect of stand on yield of forage, the data on yields in table 10 was corrected, using data obtained from a stand count made October 17, 1951 shown in table 14. Such a correction gave about the same change in the gross yields as the removal of the influence of weeds in the plots did. (See table 12.) The principal differences were the increase in the yield from the Kenland plots compared with the other varieties. This is a reflection of the much greater stand in this variety going into the second winter. The effect on the soil treatment yields was slight. However, the relationship between the yields from DDT-treated plots and that from untreated plots was reversed, with the untreated now having the lowest yield. The use of stand indices taken in October to correct yields harvested August 14 may introduce an error, owing to reduction in stand that might occur between the time of harvest and the time the stand estimates were made. These indices may account in part for the greater reduction in the yields of the untreated plots.

In order to measure the amount of deterioration of the productivity of the various treatments during the crop year, the yields on August 14 corrected for weeds were divided by the stand-corrected yields on June 12, 1951, and multiplied by 100 to obtain a ratio of the decrease in yield. These ratios are given in table 13. However, since growth conditions are less favorable in the late summer than in spring and early summer, these ratios might possibly be greater if the effect of the different growing conditions could be evaluated quantitatively. A study of these ratios shows that the Kenland variety had a notably higher ratio than the other three varieties. Wisconsin Mildew Resistant had the lowest ratio of yield although not significantly different from Midland and Michigan Common. Among the soil treatments only BHC had a considerably greater ratio than the untreated. The toxaphene and mixed insecticides had higher ratios than the untreated. All the other treatments had higher ratios than the untreated including that of DDT, owing mainly to the response of the Midland and Michigan Common varieties in these plots.

FINAL STANDS IN 1951.—The best measure of the maintenance of the clover stands is an evaluation of stands of clover at the end of the crop year and just prior to the second winter. Differences in the stand in the various insecticide plots and among the varieties of clover were visually quite prominent. Figures 1, 2 and 3 show the stand in the BHC, DDT and untreated plots where the greatest differences were observed. On October 17, stand indices were taken in each subplot using the sampling grid method. The averages of these counts are given in table 14. The stand in the Kenland variety was importantly greater than any of the other varieties, which did

Table 11.—Pounds of red clover forage (green weight) corrected for weeds present in plots 15 months after soil was treated with insecticides and fungicides prior to seeding. Lafayette, Indiana, August 14, 1951.[a]

Variety	DDT	BHC	Chlordane	Toxaphene	Parathion	Mixture	Arasan	Untreated	Variety Average
Kenland	4.4	11.6	8.8	10.0	9.1	11.4	8.0	7.5	8.8
Midland	3.1	7.3	4.8	5.9	4.9	6.9	5.3	3.9	5.3
Wis. Mildew Resistant	2.2	6.3	4.1	4.5	3.7	6.2	4.1	2.7	4.2
Mich. Common	1.1	7.0	3.9	6.2	6.2	5.0	4.9	2.6	4.6
Insecticide average	2.7	8.0	5.4	6.7	6.0	7.4	5.5	4.2	5.7

L.S.D. for odds of:	Insecticides	Varieties	Varieties for given insecticides	Insecticides for given variety
19:1	2.4	0.7	1.9	2.4
99:1	3.4	0.9	2.6	3.3

[a] Data from table 10 corrected for weeds present as represented by visual indices listed in table 9, using the regression method.

Table 12.—Pounds of red clover forage (green weight) corrected for stand on October 17, 1951, from plots 15 months after soil was treated with insecticides and fungicides prior to seeding, Lafayette, Indiana, August 14, 1951.[a]

Variety	DDT	BHC	Chlordane	Toxaphene	Parathion	Mixture	Arasan	Untreated	Variety Average
Kenland	6.2	13.4	10.9	11.9	10.0	13.3	8.0	7.4	10.1
Midland	2.3	8.5	3.4	4.5	4.8	4.6	3.7	2.0	4.2
Wis. Mildew Resistant	1.5	5.3	2.0	2.6	1.5	5.1	1.2	0.6	2.5
Mich. Common	4.4	6.0	3.7	5.7	3.4	4.9	3.9	1.3	4.1
Insecticide average	3.6	8.3	5.0	6.2	4.9	7.0	4.1	2.8	5.2

L.S.D. for odds of:	Insecticides	Varieties	Varieties for given insecticide	Insecticides for given variety
19:1	2.2	0.9	2.6	2.7
99:1	3.0	1.2	3.5	3.8

[a] Data from table 10 corrected for stand indices as given in table 13.

Table 13.—Weight of red clover forage harvested from plots with soil treated with insecticides and fungicides prior to seeding. Yields on August 14 as ratio of those harvested on June 12, 1951. Lafayette, Indiana.[a]

Variety	DDT	BHC	Chlordane	Toxaphene	Parathion	Mixture	Arasan	Untreated	Variety Average
Kenland	27.6	48.6	34.8	42.5	35.2	40.1	35.4	35.6	37.5
Midland	26.3	29.3	17.3	23.9	21.1	25.5	25.6	15.8	23.0
Wis. Mildew Resistant	11.6	25.1	16.2	18.3	13.7	24.9	15.9	11.4	17.2
Mich. Common	16.3	31.4	14.3	25.8	23.3	19.3	17.6	11.9	20.0
Insecticide average	20.4	33.6	20.6	27.6	23.3	27.4	23.4	18.7	24.4

L.S.D. for odds of:	Insecticides	Varieties	Varieties for given insecticide	Insecticides for given variety
19:1	8.8	3.4	9.8	10.6
99:1	12.2	4.6	13.1	14.4

[a] Based upon individual plot data, and averaged and analyzed.

Table 14.—Stand indices of red clover plants in plots 17 months after soil was treated with insecticides and fungicides prior to seeding, Lafayette, Indiana, October 17, 1951.[a]

Variety	DDT	BHC	Chlordane	Toxaphene	Parathion	Mixture	Arasan	Untreated	Variety Average
Kenland	12.5	25.0	24.7	23.7	21.3	24.3	20.2	18.0	21.2
Midland	5.2	21.7	10.2	12.5	12.8	12.0	10.3	7.2	11.5
Wis. Mildew Resistant	4.2	16.5	5.8	8.5	4.2	15.2	4.2	2.2	7.6
Mich. Common	10.3	15.0	11.3	15.0	9.2	14.3	8.7	11.5	11.0
Insecticide average	8.0	19.5	13.1	14.9	11.9	16.4	10.8	7.8	12.8

L.S.D. for odds of:	Insecticides	Varieties	Varieties for given insecticide	Insecticides for given variety
19:1	4.5	1.9	5.4	5.8
99:1	6.3	2.6	7.3	7.9

[a] Number of 6×6 inch squares out of 25 containing at least one red clover crown. Multiply by factor 4 to convert to per cent stand.

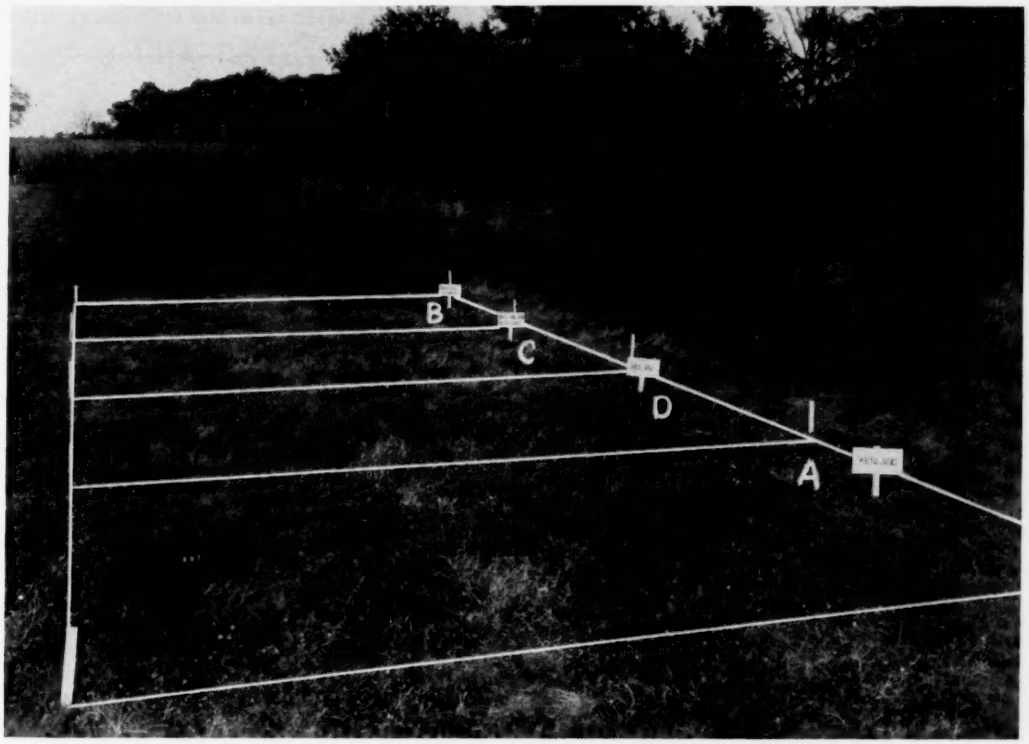

FIG. 2.—Residual stand of red clover on October 18, 1957, in soil treated with DDT prior to seeding, at end of first crop year. A—Kenland, B—Wisconsin Mildew Resistant, C—Michigan Common, D—Midland.

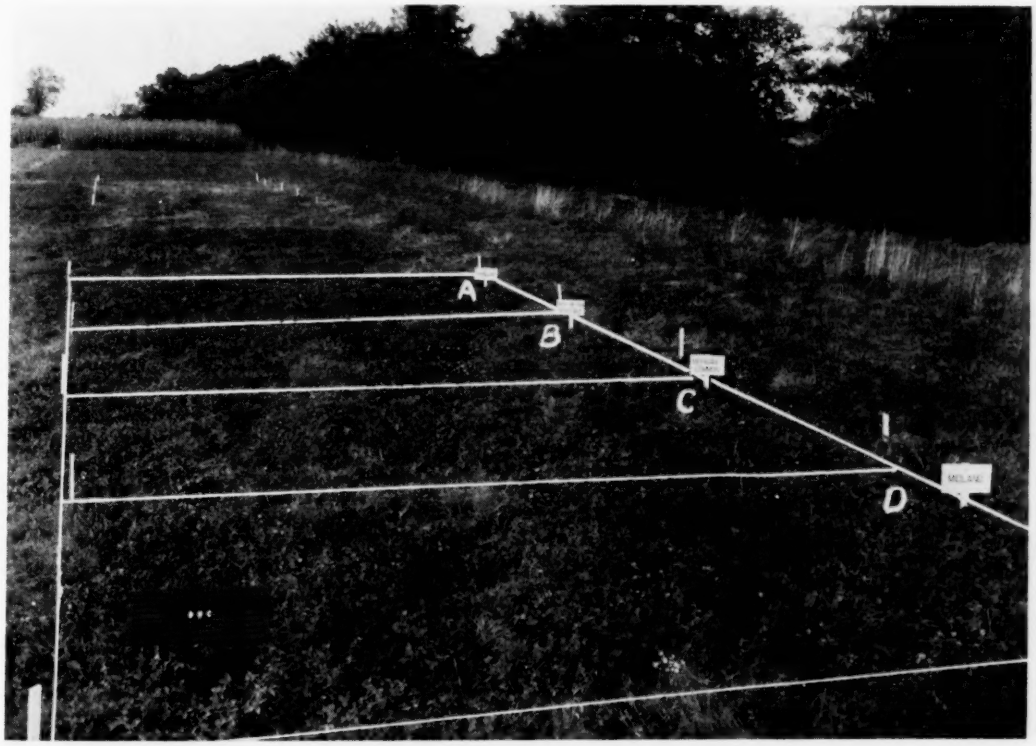

FIG. 3—Residual stand of red clover on October 18, 1957, in soil treated with BHC prior to seeding, at end of first crop year. A—Kenland, B—Wisconsin Mildew Resistant, C—Michigan Common, D—Midland.

Table 15.—Summary of data from plots of red clover with soil treated with insecticides and fungicides prior to seeding Lafayette, Indiana, 1950–1951.

Source Table	Data	Soil Treatments								Red Clover Varieties			
		DDT	BHC	Chlordane	Toxaphene	Parathion	Mixture	Arasan	Untreated	Kenland	Midland	Wis. MR.	Mich. common
1	Plants/sq. ft.(1950)	5.1	*16.0*	13.0	12.2	8.6	12.7	11.4	11.0	*12.4*	11.6	11.1	10.2
3	Weeds/sq. ft. (1950)	71.3	*31.8*	63.6	54.4	54.9	44.5	75.1	79.4	61.8	59.4	*56.0*	60.2
4	Stand-visual rating April 30, 1951	1.2	*8.0*	4.7	5.6	4.6	6.6	4.4	4.7	*5.7*	5.2	5.1	4.0
5	Stand index, 4/30[a]	10.0	*24.1*	22.4	22.0	20.4	22.4	20.9	19.6	*21.6*	20.6	20.3	18.4
6	Weight forage 6/12	13.6	24.2	25.8	24.4	26.0	*26.7*	23.8	22.6	23.2	23.3	*24.5*	22.4
7	Weight forage 6/12 corrected for stand	6.1	23.4	23.1	21.4	21.7	*24.0*	20.0	18.0	*20.6*	20.1	20.4	17.6
9	Weed estimates 8/14	7.7	*2.3*	4.2	3.2	4.2	2.8	4.2	5.1	*2.6*	4.2	4.8	5.2
10	Weight forage 8/14	*11.0*	10.4	8.8	9.8	9.8	10.1	9.4	8.2	*11.9*	9.2	8.1	9.6
11	Weight forage 8/14 corrected for weeds	2.7	*8.0*	5.4	6.7	6.0	7.4	5.5	4.2	*8.8*	5.3	4.2	4.6
12	Weight forage 8/14 corrected for stand on October 17	3.6	*8.3*	5.0	6.2	4.9	7.0	4.1	2.8	*10.1*	4.2	2.5	4.1
13	Stand index 10/17[a]	8.0	*19.5*	13.1	14.9	11.9	16.4	10.8	7.8	*21.2*	11.5	7.6	11.0
14	Ratio 8/14 yield to yield June 12	20.4	*33.6*	20.6	27.6	23.3	27.4	23.4	18.7	*37.5*	23.0	17.2	20.0

[a] Multiply by the factor 4 to obtain per cent stand. Italicized data indicate the treatment ranking first or best in that particular category. For significant differences see preceding tables.

not differ significantly from each other, although Wisconsin Mildew Resistant had the poorest stand. Among the soil treatments only the BHC and mixed insecticides had considerably better residual stands than the untreated. The other treatments did not differ greatly from the untreated, although the DDT plots had the fewest remaining plants. In studying the effect of the BHC treatment on the individual varieties, there were marked differences in the response. In the BHC plots, Kenland had 1.4 times, Midland 3.0 times, Wisconsin Mildew Resistant 7.5 times, and Michigan Common 1.3 times more plants than the untreated. The BHC treatment apparently resulted in conditions more favorable to the maintenance of clover stands than the other insecticides, and this effect was more pronounced in those strains more susceptible to the unfavorable conditions. A similar relationship existed to a lesser degree in the mixed insecticide plots, although the Midland variety had no better stand than in the other treatments except DDT. Michigan Common response to the toxaphene treatment was similar to the BHC and mixed insecticides, but was like the untreated in all the other treatments including DDT. The stand of Wisconsin Mildew Resistant variety showed little evidence of stand maintenance other than that exhibited in the BHC and mixed insecticide treatments. This variety had higher residual stands in all treatments than in the untreated with the best stand in the toxaphene plots, where it had approximately four times as many plants as in the untreated.

Table 15 gives a summary of the data for all soil treatments and varieties of red clover.

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Injury of Clover Root Curculios to Red Clover in New Jersey[1]

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ABSTRACT

A 3-year survey of red clover fields in New Jersey showed that high populations of *Sitona hispidula* (F.) and *S. flavescens* Marsham were present throughout the State and extensive larval feeding was observed on the roots of seedling and second-year stands. Sitonids were found, by the use of a soil-filtering process, to be the most numerous of all insects attacking red clover roots.

During 1956 and 1957 the average number of feeding scars produced on roots by sitonids during the larval period was 1.18 and 1.26, respectively, or an overall average of 1.22 feeding scars per sitonid. Using this figure to estimate sitonid larval populations from feeding-scar data in plots that were sampled after the sitonid larvae had completed their development and had emerged from the soil as adults, populations of 50 or more per square foot were not unusual and populations as high as 124 per square foot have been found. Estimated populations on a Statewide basis averaged about 73 sitonids per square foot.

In recent years red clover has become undependable as a crop in New Jersey, as in other parts of the United States. Many factors are believed to contribute to this; among them are fertility of the soil, soil moisture, cultural practices, weather, plant diseases, nematodes, and insects. These factors appear to vary in importance, depending on local conditions.

A survey of 32 seedling red clover fields in New Jersey in the fall of 1955 showed that insect feeding scars were present on the roots. Six samples had no insect injury, 6 had insect feeding scars on all their roots, 10 had insect feeding scars on 60 to 90% of the roots, and 10 had insect feeding scars on 20 to 50% of the roots.

Of the 23 survey samples checked for internal root necrosis, 20 were positive and 4 showed internal necrosis on 100% of the roots. Of the 17 survey samples checked for nematodes in the roots, 15 were positive and all had nematodes in the soil. The nematodes found in the root and soil samples were considered plant parasitic.

A soil and plant survey of 13 seedling and 31 second-year red clover fields was made in the early summer of 1956 to determine the number and kinds of insects associated with the roots of red clover. Sitonid larvae represented 51% of the total 1,467 insects collected and 78% of the insects with chewing mouth parts that might produce feeding scars on the roots. A breakdown of the insects and other arthropods found in this survey showed a similar distribution of insects in the seedling and second-year fields, but greater numbers of the organisms occurred in the second-year red clover fields. Sitonids were considered responsible for most of the feeding scars on the roots of red clover in New Jersey.

During the course of this study many adult sitonid beetles were collected and of those checked for identification 83% were *Sitona hispidula* (F.) and the rest were *Sitona flavescens* Marsham. Two other species of *Sitona* reported present in the Northeastern United States, *S. cruiatus* Gyllehal and *S. cylindricollis* Fahr., were not found in this study.

LIFE HISTORY OF SITONIDS.—*Sitona hispidula* and *S. flavescens* were found to have a one-generation-per-year life cycle in New Jersey. Adults mate and lay eggs in the fall, overwinter in the fields under surface debris, and resume egg laying in the spring. The bulk of the overwintering adults died in late May. The larval stage can be found in the soil during April, May, June, and July, with the pupal stage occurring early in June and the new adults emerging the latter half of June.

Egg laying was observed in the fall and spring and under laboratory conditions the eggs hatched in approximately 3 days. Forty beetles observed for egg deposition lived from a few days to a month. Two beetles that produced eggs for the longest period laid 396 and 271 eggs, respectively, for an average of 13 and 12 eggs per day.

SAMPLING.—To determine the extent of sitonid injury to red clover roots, sampling consisted of a collection of 20 or more roots dug at random from a field. They were shaken free of soil and placed in a plastic bag to be returned to the laboratory for processing. To obtain counts of the larval population in a field, soil and plant samples were taken, each sample containing at least one plant to the depth of the roots. Three such samples were taken per field or plot to obtain a sample of approximately 1 sq. ft. Samples were placed in plastic bags and tagged for laboratory processing.

Soil and plant samples were processed by running the soil through screens with water to screen out insects and closely related organisms. The mesh sizes of the screens were $\frac{1}{2}''$, $\frac{1}{4}''$, $\frac{1}{8}''$, $\frac{1}{16}''$, $\frac{1}{32}''$, and the smallest screen with holes of 1/50 by 1/40 inch. Three such sets of six screens with wooden frames were used, each 16 inches square. Each $\frac{1}{3}$-square-foot plant sample and soil sample was distributed on the three sets of screens. Through a hand-adjusted nozzle, water washed the soil off the roots and through the screens. Then the screens were inspected for specimens of insects or closely related organisms that might be held on the screens. Insect feeding-scar counts were then taken on the external root surface of the samples as another indication of the presence and abundance of insects and as possible means of entry for disease organisms. Feeding scars were identified by the distinct margins, the hollowed-out centers, and the irregular position of the scars on the root surface. When extensive feeding resulted in a long groovelike feeding scar, the width was arbitrarily divided into the length of the groove and the quotient recorded as the number of insect-feeding scars. Any rotted area or doubtful injury was not counted as an insect-feeding scar. Only roots $\frac{1}{2}$ inch or more in diameter were examined for scars.

That the obtaining and processing of root and soil samples is so time consuming is unfortunate. It would be

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Table 1.—Second-year red-clover studies.[a]

Counties (N.J.)	No. of Fields	Date of Sampling (1956)	No. of			Estimated No. of Sitonids[b]
			No. of Roots	Insect Feeding Scars	Sitonids Found	
Somerset	1	6/4	20	92	82	75
	2	6/4	13	100	93	82
	3	6/8	5	72	57	59
Hunterdon	1	6/8	8	69	27	57
	2	6/8	10	74	88	61
	3	6/8	5	26	23	21
	4	6/8	8	10	8	8
	5	6/8	5	70	55	57
Middlesex	1	6/13	5	27	31	22
	2	6/15	5	65	26	53
Monmouth	1	6/15	5	11	59	9
	2	6/15	3	60	1	49
Burlington	1	6/15	4	46	5	38
Hunterdon	6	6/19	9	78	9	64
Somerset	4	6/19	7	87	68	71
	5	6/19	14	102	16	84
Warren	1	6/27	7	148	3	121
	2	6/27	13	144	4	118
	3	6/27	28	174	3	143
	4	6/27	9	70	18	57
	5	6/27	10	99	19	81
Mercer	1	6/28	6	93	3	75
	2	6/28	12	298	0	244
	3	6/28	7	48	0	39
	4	6/28	11	114	3	93
	5	6/28	6	34	14	28
	6	6/28	7	121	32	99
Camden	1	7/11	3	37	6	30
	2	7/11	3	55	4	45
Monmouth	3	7/17	3	216	15	177
Salem	1	7/17	4	122	1	100
Burlington	6	7/27	3	82	2	67
Totals			258	2,844	775	2,327
Average						72.7

[a] Sitonid populations and number insect feeding-scars per root. Based on three soil and plant samples 7 inches square, approximately 1 square foot, per field, consisting of 32 fields in nine counties.

[b] Using 1.22 insect feeding-scars per larva.

desirable to sample all plots at the same time, and in some cases to sample the same plot at designated intervals to obtain more detailed information on larval feeding and development. In this study it seemed more desirable to obtain information on larval abundance from a representative number of fields throughout the State than to obtain detailed information from a few fields. As all fields could not be sampled and processed before the sitonids had completed their larval period, it was desirable to establish the average number of feeding scars produced by each larva, so that the feeding-scar data could be used to estimate the larval populations of the plots processed late in the season when many of the larvae would have pupated and left the soil. To establish the number of feeding scars produced by each sitonid larvae, the sampling of plots was delayed until most of the larvae were approaching the pupal stage. At this period it was assumed that all the larvae would still be in the soil and their feeding nearly completed; thus a more accurate ratio of feeding scars to sitonid larvae could be obtained. Later sampling would allow time for some of the sitonids to complete pupation and emerge from the soil as adults, which would result in a higher ratio of feeding scars per sitonid.

In the earliest period of sampling, June 4 through June 8, 1956, samples from eight fields in Hunterdon and Somerset Counties were processed. A few pupae were observed in these samples, which fact indicates that the larval period of the sitonids was about completed. The data from these eight fields show that the sitonid population ranged from 8 to 93 with an average of 54 per square foot. The number of feeding scars per sitonid ranged from 0.84 to 2.56 with an average of 1.18. In six of the eight fields sampled the feeding scars per sitonid ranged from 1.08 to 1.27. Based on 1956 data it appears that each sitonid larva produces approximately 1.2 feeding scars on the larger roots during the larval period.

Data from some check plots processed on June 8, 1957, show an average of 1.26 insect feeding scars per sitonid. Data from 1956 and 1957 indicate that the sitonid larva does a limited amount of feeding on the larger roots and is responsible for approximately 1.22 feeding scars during the larval period. This figure of 1.22 feeding scars per sitonid may seem to show very little feeding for a larva during a feeding period that extends over several weeks. However, only roots of $\frac{1}{8}$ inch or more in diameter were checked for scars. When these larvae are small they are known to feed on the nodules containing the nitrification bacteria, and as they grow they feed on fine roots working their way to the main root (Bigger 1930). The larvae would, therefore, probably be of fairly good size before making abrasions that would be recorded on the roots of $\frac{1}{8}$ inch, or more, diameter. This figure of 1.22 feeding scars per larva can be used to estimate the sitonid populations from insect-feeding-scar data in plots processed later in the season when the sitonids have completed their larval feeding period and many individuals have pupated and emerged from the soil as adults. Table 1 gives observed and estimated larval populations for all plots sampled in this study.

Based on the number of sitonids recovered from the earliest plots sampled, a high sitonid population was apparently present in red clover fields in New Jersey in 1956. Samples approximately 1 foot square yielded 93, 88, 82, 68, 59, and 55 sitonid larvae, which represent populations ranging from 2,395,800 to 4,051,080 sitonids per acre. The 1957 data show a sitonid population as high as 5,401,440 per acre.

The estimated sitonid populations based on the observed feeding-scar data show that certain of the plots sampled during the later periods of this study had higher sitonid populations than those sampled earlier. The highest estimated population of sitonids in these plots was 244 per square foot or 10,628,640 per acre. When sitonid populations are checked against field locations it is apparent that high sitonid populations are found throughout the State.

During the 1956 and 1957 seasons, 50 seedling red clover fields were surveyed for sitonid injury to the roots. Sitonid injury was found in 84% of the fields and in 15% of the roots. The average number of feeding scars per injured root was 3.0 and for all roots 0.64. A similar survey of 66 second-year red clover fields showed sitonid injury in all fields. The number of feeding scars per root ranged from 1 to 25 and averaged 6.9 per root.

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Effect of Repellents on the Evolution of Carbon Dioxide and Moisture from Human Arms[1]

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ABSTRACT

Repellents applied to the arms of three subjects did not affect the evolution of moisture, but dimethyl phthalate, diethyltoluamide, and ethyl hexanediol did reduce the carbon dioxide from two of the subjects and diethyltoluamide reduced it from the other. Production of carbon dioxide was correlated directly, and production of moisture inversely, with natural attractiveness in the absence of repellents, but natural attractiveness was not correlated with the protection obtained with repellents.

Carbon dioxide and moisture have long been of interest as mosquito attractants, but the results of studies by different workers have not agreed in all respects. According to Rudolfs (1922) carbon dioxide was not an attractant but an activator, and Willis (1947) considered it not an attractant at concentrations of 1%, 10%, and 50% in air. Thompson & Brown (1955) reported that it was probably not the attractant in human sweat, although it did increase the attractiveness of water-soaked cloth. Experiments with field traps containing dry ice indicated that carbon dioxide was attractive to mosquitoes (Headlee 1941, Huffaker & Back 1943). Tests by Parker (1948) and field tests with robots by Brown (1951) also suggested that moisture and carbon dioxide were attractive. Most of these tests were conducted with olfactometers or inanimate objects. In tests with human subjects Brown (1958) observed that the hands of individuals with low moisture output were more attractive than those with high moisture and that warm skin was more attractive than cool.

The effects of repellents on the evolution of carbon dioxide and moisture from human arms, and the correlation of this evolution with the natural attractiveness of human beings and the protection time of repellents were studied at Orlando, Florida.

MATERIALS AND METHODS.—Three white male subjects, 33 to 35 years old and weighing 170 to 175 pounds, took part in the tests. The repellents used were dimethyl phthalate, diethyltoluamide (85% meta and 15% para isomers), and ethyl hexanediol. Determinations were made of the amounts of carbon dioxide and water liberated from measured areas of the forearms (394 to 537 sq. cm.) when untreated and when treated with the repellents. The hands were covered with rubber gloves, sealed to the wrists with adhesive tape.

Ethanol solutions of the repellents were applied from a pipette and spread with a glass rod. The quantity applied was determined by spectrophotometric measurements of the amount delivered by the pipette and the amount recovered from the glass rod by rinsing with ethanol. The treated area was slightly smaller than the exposed area to prevent the rubber gloves from touching the repellent. The treated arms were immediately exposed to caged 7- to 8-day-old mosquitoes for 1 minute, to be sure the repellents were effective at the applied dose.

In order to collect samples of carbon dioxide for analysis, the treated and untreated arms were sealed in stainless-steel frustum-shaped vessels by means of rubber sleeves taped to the outside of the vessel and to the upper arm. The vessels were 15 inches long, tapering from 9 to 3½ inches in diameter, and enclosing a volume of 4,500 ml. Each vessel had an opening in the small end and another in the side, near the large end. These openings could be closed with perforated rubber stoppers. Tubing, passed through the stoppers, provided a means of passing gas through the system.

The vessels were flushed for 3 minutes with dry, carbon-dioxide-free nitrogen at the rate of 6 liters per minute to remove carbon dioxide that had accumulated during the enclosure of the arms. The rubber tubing at the small end of each vessel was then closed with a screw clamp and the stopper near the large end was replaced with a thermometer. The temperatures of the room and of the gas within the vessels were recorded at various intervals. Room temperature was maintained at 27° ± 2° C. and the temperatures inside the vessels approximated 32° throughout the tests. The subjects were kept in a reclining position during the collection period.

Samples of the gas were taken after the arms had remained in the vessels for 4 hours. The sampling apparatus consisted of a 625-ml. gas collector connected to a 1,000-ml. leveling bulb. This system contained approximately 800 ml. of saturated sodium chloride solution (Treadwell & Hall 1945). The tube in the small end of the vessel was connected to the gas collector, and the solution therein was displaced with gas from the vessel. It was necessary to apply vacuum at the top of the leveling bulb to facilitate the displacement.

In the process of sampling, the rubber sleeve was drawn into the vessel by the vacuum, leaving a concave surface at the large end which caused a decrease in the volume of the vessel. This change in volume was measured by the amount of water required to fill the concavity. The gas in the collector was transferred to a 1,000-ml. round-bottomed flask containing 25 ml. of 0.1 *N* barium hydroxide at 40 to 50 mm. pressure. The flask was shaken frequently during an interval of 1 hour; then the excess barium hydroxide was titrated under nitrogen to the phenolphthalein end point with 0.1 *N* hydrochloric acid (1 ml. is equivalent to 1.12 ml. of CO_2 at s.t.p.). The volume of carbon dioxide in the sample was calculated from the amount of barium hydroxide consumed, and converted to Q, the milliliters of carbon dioxide evolved per 100 sq. cm. of skin surface per hour, by the equation:

$$Q = \frac{(Va+Vb)-(Vc+Vd)}{4aVb} \times 100\ Qa$$

where Va, Vb, Vc, and Vd are the volumes (ml.) of the vessel, gas collector, hand plus arm, and concavity in the rubber sleeve respectively; A is the area of exposed skin

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Table 1.—Carbon dioxide emitted from test and check arms of three subjects, and the amount of repellent recovered by rinsing the test arm with ethanol.[a]

Treatment on Test Arm	Carbon Dioxide (Ml./100 Sq. Cm./Hr.)		Repellent		
			On Arm (Mg./100 Sq. Cm.)		Recovered in Vessel (Mg.)
	Test Arm	Check Arm	Applied	Recovered	
Right Arm Treated					
Subject A:[b]					
Untreated	1.68	1.73	—	—	—
Dimethyl phthalate	1.58	1.84	89	72	4
Diethyltoluamide	1.65	1.91	82	58	4
Ethyl hexanediol	1.64	1.90	67	53	6
Subject B:					
Untreated	1.91	1.94	—	—	—
Dimethyl phthalate	2.04	2.24	90	62	20
Diethyltoluamide	1.83	2.00	74	46	12
Ethyl hexanediol	1.85	2.09	70	48	12
Subject C:					
Untreated	1.58	1.52	—	—	—
Dimethyl phthalate	1.60	1.60	93	55	45
Diethyltoluamide	1.31	1.45	86	59	18
Ethyl hexanediol	1.50	1.48	69	40	10
Left Arm Treated					
Subject A:					
Untreated[b]	1.73	1.68	—	—	—
Dimethyl phthalate	1.74	1.73	106	71	4
Diethyltoluamide[c]	1.71	1.73	89	64	3
Ethyl hexanediol	1.56	1.51	70	52	8
Subject C:					
Diethyltoluamide	1.41	1.55	89	61	2

[a] 2 tests of 4 hours each except where indicated.
[b] 4 tests.
[c] 3 tests.

(sq. cm.), and Qa the milliliters of carbon dioxide in the sample.

In the moisture tests the procedure for enclosing and treating the arms was the same as in the carbon dioxide tests except that the vessels were flushed with dry nitrogen at the rate of 6 liters per minute for 15 minutes to remove the moisture that had accumulated during enclosure of the arms. Each vessel was then connected to a tared Schwartz drying tube, filled with indicating anhydrous calcium sulfate, that was protected by another drying tube from any back flow of atmospheric moisture. The flow of dry nitrogen was reduced to 1 liter per minute, measured by a flowmeter at the beginning of the system; another flowmeter at the end gave the same reading, indicating that the system was airtight. After a collection period of 1 hour the tared drying tube was disconnected and weighed; the increase in weight was calculated as moisture.

When the collections of carbon dioxide or water had been completed, the treated arms were exposed to mosquitoes to determine whether the amount of repellent remaining was still effective. The arms and vessels were then rinsed with distilled ethanol and the amounts of repellent recovered were determined spectrophotometrically by the methods of Schmidt *et al.* (1958) for diethyltoluamide and of Bowman *et al.* (1959) for ethyl hexanediol; dimethyl phthalate was read at 225 mμ.

The first collections were made to compare the carbon dioxide or water liberated from the two arms of each subject simultaneously when neither arm was treated with repellent. Later a treated and an untreated arm were exposed simultaneously.

Results and Discussion.—The quantities of carbon dioxide evolved from untreated and repellent-treated arms are given in table 1. Although the carbon dioxide from the arms of all subjects varied from day to day, the quantities from the two arms when both were untreated in any given test agreed within 4%. Shaw & Messer (1930) showed that the quantity of carbon dioxide evolved from human skin varied directly with the relative humidity of the surrounding gas when the temperature was constant. Since in these tests the arms were enclosed in airtight vessels for 4 hours at 32° C, the relative humidity of the gas inside the vessels was assumed to approach 100% within the first hour and thereafter the maximum yield of carbon dioxide at this temperature would be obtained.

When the right arm was treated, all three repellents reduced the carbon dioxide emitted by subjects A and B by 9% to 14%, whereas subject C was affected only by diethyltoluamide, which gave about a 10% reduction. Although these reductions are considerably greater than the differences between untreated arms (4%), they are not great enough to indicate that the mode of action of these repellents is based upon the retardation of carbon dioxide evolution.

When the left arm of subject A was treated, the quantity of carbon dioxide evolved from both arms was essentially the same, but when the left arm of subject C was treated with diethyltoluamide the carbon dioxide was diminished by the same amount as when his right arm was treated. The reason for the inconsistent behavior of subject A is not understood; however, these data offer further evidence that retardation of carbon dioxide evolution has little relation to the action of repellents.

The quantities of moisture collected from untreated and repellent-treated arms are given in table 2. As in the carbon dioxide tests, the quantities from the arms of all subjects varied from day to day, but in most individual tests the two arms agreed within about 5%, indicating that no real difference in the amount of moisture evolved was caused by application of the repellent.

In all tests the amount of repellent recovered from the arms at the end of the exposure period was less than that applied; the difference was attributed to absorption and evaporation. In the carbon dioxide tests (table 1) subject C lost more repellent than B, who lost more than A, but

Table 2.—Moisture emitted from test and check arms and the amount of repellent recovered.[a]

Treatment on Test Arm	Water (Mg./100 Sq. Cm./Hr.)		Repellent		
			On Arm (Mg./100 Sq. Cm.)		Recovered in Vessel (Mg.)
	Test Arm	Check Arm	Applied	Recovered	
Subject A:					
Untreated	117	120	—	—	—
Dimethyl phthalate	115	119	80	76	2
Diethyltoluamide	149	144	83	74	1
Ethyl hexanediol	128	134	69	61	2
Subject B:					
Untreated	113	107	—	—	—
Dimethyl phthalate	128	122	95	81	1.3
Diethyltoluamide	127	111	74	62	0.3
Ethyl hexanediol	127	120	66	61	2.3
Subject C:					
Untreated	170	154	—	—	—
Dimethyl phthalate	138	134	78	75	16
Diethyltoluamide	155	152	81	76	6
Ethyl hexanediol	134	139	72	64	2

[a] 2 tests of 1 hour each.

Table 3.—Biting rates of *Aedes aegypti*, evolution of carbon dioxide and moisture from untreated arms of three subjects, and protection times with three repellents.[a]

Subject	Bites/Sq. In./Min.[b]	Carbon Dioxide (Ml./100 Sq. Cm./Hr.)	Moisture (Mg./100 Sq. Cm./Hr.)	Protection Time (Minutes)		
				Dimethyl Phthalate	Diethyl-toluamide	Ethyl Hexanediol
A	0.64	1.70	118	76	202	192
B	2.01	1.92[c]	110[c]	48	232	200
C	.22	1.55[c]	162[c]	18	105	85

[a] 4 tests except where noted.
[b] 12 tests.
[c] 2 tests.

in the moisture tests (table 2) the recovery from all subjects was essentially the same. With the exception of dimethyl phthalate on subject C, the repellent remaining after the tests protected all subjects against mosquitoes.

Data on the natural attractiveness of the subjects, as shown by the biting rates on untreated arms, and the protection times provided by the repellents in other tests are shown in table 3. Subject B, who had the highest natural attractiveness, evolved the most carbon dioxide and the least moisture, while C, who had the lowest attractiveness, evolved the least carbon dioxide and the most moisture. These results are in line with the data obtained by Brown (1958). Neither the attractiveness of the subjects nor the evolution of carbon dioxide or moisture appeared to be correlated with the protection time, which is believed to be governed by the loss of repellent from the skin caused by absorption and evaporation.

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Effect of Soils upon the Uptake of Systemic Insecticides by Plants[1]

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ABSTRACT

The introduction of organic systemic insecticides has led to a number of new methods of application for control of foliage-feeding insect pests, among which, various types of soil treatment are of importance. In evaluating the efficiency of soil treatments in the field it has been noted that the effectiveness of these applications often depends upon soil conditions. Therefore, experiments were conducted to determine 1) the effect of various soil types upon systemic insecticide absorption by plants from the soil, and 2) the characteristics of soil which bind the insecticides against the leaching action of water.

The absorption of some systemic insecticides by peas from four soils and two sands was measured by aphid bioassay and anticholinesterase analysis. Greater residues and longer periods of insect kill resulted from soil drench applications to sands and sandy soil than from applications to other soils (silt loam, clay loam, and muck). Pea aphid (*Macrosiphum pisi* (Harris)) control from soil treatments with Phosdrin varied from 2 days on plants grown in loam and muck to 19 days on plants in sand. Likewise, demeton and phorate, formerly designated Thimet® (*O,O*-diethyl *S*-(ethylthio)methyl phosphorodithioate), were most effective in the sands and least effective in the muck, with insect kill from 10 to 100% occurring on peas in all soils and sands at 41 days after treatment. Schradan was also more effective in treated sands and sandy soils. Because of its slow toxicological action schradan provided low insect mortality when counts were taken 24 hours after the insects were subjected to treated plants. Unlike the other insecticides, Isolan® (dimethyl 5-(1-isopropyl-3-methyl-pyrazolyl) carbamate) was absorbed in greater amounts by peas from the muck than from either of the loam soils. Anticholinesterase analysis of pea plants from Isolan and Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate, 60% technical) treated soils gave results comparable with those obtained by insect bioassay.

The binding of an insecticide in soil was studied by leaching radioactive Phosdrin through columns of 12 soils. The largest amounts of Phosdrin were bound by peat (127 micrograms per 100 grams of soil), with less being bound by heavy loam soils (10 to 27 micrograms per 100 grams of soil) and the least by sandy soils (3 to 8 micrograms per 100 grams of soil). The amount of Phosdrin bound by the various soils correlated in a positive manner with the base exchange capacity, organic matter content and nitrogen content, but it was concluded that the organic matter content was primarily responsible for insecticide binding. Other chemical and physical properties of the soil did not correlate with the binding of Phosdrin.

Soil applications of systemic insecticides offer a promising method of treatment particularly because of their long residual action. The efficiency of a soil treatment with systemic materials depends upon the plant's ability to absorb and translocate the toxicant to the foliage. Soil conditions and soil characteristics greatly affect the absorption of insecticides by plants.

Greater insecticide absorption occurs from sands and sandy soils than from loams, clays and muck soils (David & Gardiner 1951, David 1951, Davich 1953, and others). David (1951) measured the absorption of radioactive schradan by beans from sand and soil and observed greater uptake from the sand. He concluded that schradan was less readily absorbed from the soil because the insecticide was held in the top layers by adsorption and could not be reached by the plant roots. Greater absorption of schradan occurs from sands than from soils partly because plants grown in sand have a more extensive root system than plants grown in soil (Wallace 1951). David (1957) observed that transplanted broadbeans absorbed less demeton than nontransplanted beans, but whether the beans were transplanted or not, greater absorption always occurred from the sand.

Organic matter appears to be the primary factor concerned in the reduced effectiveness of hydrocarbon insecticides against many soil insects. By using a fly bioassay technique, Edwards *et al.* (1957) found a correlation between organic matter content and insect kill when working with aldrin and lindane in the soil. It appeared that the insecticide was adsorbed on the organic matter, thus reducing its effectiveness. A similar correlation was found to exist in the field between organic matter and percent control of root maggots on rutabagas (Forbes & King 1957).

Although experimental reports are lacking it appears that organic matter plays an important role in the reduced absorption of systemic insecticides by plants. Soils with more organic matter and smaller particle size reduced the rate of absorption of schradan by peas as indicated by bioassay (Casida *et al.* 1952). Johanson (1952) also stated that high organic matter containing soils decreased the rate and amount of absorption of demeton and schradan by plants.

In evaluating the efficiency of various soil treatments it has been noted that the effectiveness of these treatments often depends upon the soil type. Therefore, experiments were conducted in the laboratory to determine 1) the effect of various soils upon systemic toxicant absorption by plants, and 2) the properties of the soil which bind the insecticide and reduce leaching and plant absorption of the insecticide.

ABSORPTION OF SYSTEMIC INSECTICIDES FROM SOILS AND SANDS BY PEAS.—*Bioassay.*—The absorption of five systemic insecticides (Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate, 60% technical), schradan, phorate(formerly designated Thimet®, *O,O*-diethyl

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Table 1.—Characteristics of the soils used for measuring absorption of systemic insecticides by pea plants.

Soil	Per Cent						Pounds per Acre		
	Sand	Silt	Clay	O.M.[a]	B.E.C.[b]	pH	N	P	K
Quartz sand	100	0	0	0.0	0.0	8.5	0	0	0
Beach sand	91.0	1.0	8.0	0.6	0.4	7.5	10	150	20
Plainfield sand	83.0	8.0	9.0	1.2	4.3	5.3	175	170	145
Superior clay loam	40.0	36.0	24.0	2.8	12.8	7.0	225	40	170
Clyde silt loam	4.0	76.0	20.0	3.6	32.3	6.8	400	400	930
Muck	78.0	11.0	11.0	54.0	65.0	6.2	800	300	1050

[a] Organic matter.
[b] Base exchange capacity expressed as milliequivalents per 100 grams of soil.

S-(ethylthio) methyl phosphorodithioate), Isolan® (dimethyl 5-(1-isopropyl-3-methyl-pyrazolyl) carbamate) and demeton) by peas from four different soil types and two sands was determined by bioassay with pea aphids (*Macrosiphum pisi* (Harris)). The soils and their characteristics are given in table 1.

Peas (Little Marvel variety) were planted in each of four soils and two sands contained in No. 2 cans. Twenty-four hours before the insecticides were applied, 50 ml. of water were added to each can. This provided a condition almost equivalent to field capacity (the amount of water held in a soil after it has been saturated and allowed to drain for 24 hours) at the time the soil was treated. After insecticide applications were made, wax paper covers were placed over the soil to prevent volatilization which could cause a fumigant action upon the insects.

Three- to five-inch pea plants (four per can in 42 cu. in. of soil) were treated with 18.75 micromoles of the insecticide in 25 ml. of water as a soil drench. All treatments were replicated four times. Fifteen insects (fourth- and fifth-instar pea aphids) were placed on the plants of each can and mortality counts were taken 24 hours later. After counting, the remaining insects were removed. Twenty-four hours before the next count, a new group of 15 insects was introduced on each treatment. Counts were taken 1, 2, 3, 5, 9, 17, 25, 33, and 41 days after treatments, or until no further toxicity was evident.

Phosdrin gave 100% kill in all materials 24 hours after treatment and was absorbed from quartz sand in sufficient quantities to give effective residual kill for 19 days (table 2). (In this discussion, effective residual kill will be designated as the number of days in which 50% or greater mortality was obtained.) The period of effective kill from plants grown in beach sand was only 8 days, less than half of that for the quartz sand. Phosdrin in the Plainfield sand gave effective residual mortality for 3.3 days while in the heavier soils it persisted for 2.5 days or less, being least effective in Superior clay loam (1.5 days).

Very little or no kill resulted 24 hours after treatment with schradan from any soil material except the quartz sand. Schradan gave its greatest percentage of mortality 5 days after treatment in all soils. The most efficient insect control was obtained with plants grown in quartz and beach sand. Peas grown in the agricultural soils did not absorb enough schradan to provide 100% mortality in a 24-hour period at any time throughout the duration of the test. The most efficient insect control resulted from the Plainfield sand (95% mortality) and to a lesser extent from the Superior clay loam (71% mortality) 5 days after treatment. Insect kill was very poor from peas grown in muck and Clyde silt loam. These soils gave a maximum mortality of only 49% and 54%, respectively.

Isolan, Thimet and demeton gave 100% control or nearly that for the duration of the experiment in the three sands. These three insecticides were absorbed more slowly from muck and silt loam than from the other four materials as indicated by insect mortality 24 hours after treatment. Thimet gave more than 95% control 41 days after treatment in the silt and clay loams while the mortality in muck was only 37%. Insect kill from demeton was not as high as the kill from Thimet towards the end of the test. The effective residual period of demeton was 25 days with plants grown in muck and 41 days in clay loam. Forty-one days after treatment 63% mortality was obtained from the peas in Clyde silt loam with demeton. Unlike Thimet and demeton, Isolan gave

Table 2.—Absorption of systemic insecticides from different soils as indicated by pea aphid mortality on peas.

Soil	Per Cent Control After Days Indicated								
	1	2	3	5	9	17	25	33	41
				Phosdrin					
Quartz sand	100	100	100	100	100	59	25	0	—
Beach sand	100	100	100	100	13	0	—	—	—
Plainfield sand	100	97	64	8	0	—	—	—	—
Superior clay loam	100	27	12	0	—	—	—	—	—
Clyde silt loam	100	88	26	0	—	—	—	—	—
Muck	100	59	10	0	—	—	—	—	—
				Schradan					
Quartz sand	62	98	93	100	100	92	48	51	33
Beach sand	9	82	84	100	97	61	53	49	19
Plainfield sand	4	48	72	95	92	37	35	37	5
Superior clay loam	0	29	29	71	56	18	25	32	—
Clyde silt loam	0	4	8	54	23	0	—	—	—
Muck	0	4	4	49	23	4	0	—	—
				Phorate					
Quartz sand	100	100	100	100	100	100	100	100	100
Beach sand	100	100	100	100	100	100	100	100	100
Plainfield sand	100	100	100	100	100	100	100	100	100
Superior clay loam	100	100	100	100	100	100	100	96	98
Clyde silt loam	98	100	100	100	100	100	100	100	98
Muck	91	100	100	100	100	100	90	79	37
				Demeton					
Quartz sand	100	100	100	100	100	100	100	100	100
Beach sand	100	100	100	100	100	100	100	100	100
Plainfield sand	100	100	100	100	100	100	100	98	98
Superior clay loam	100	100	100	100	100	91	73	65	49
Clyde silt loam	98	100	100	100	100	100	95	82	63
Muck	93	100	100	100	100	80	50	35	17
				Isolan					
Quartz sand	100	100	100	100	100	100	100	100	100
Beach sand	100	100	100	100	100	100	100	100	100
Plainfield sand	100	100	100	100	100	100	100	100	100
Superior clay loam	100	100	100	100	100	100	95	77	44
Clyde silt loam	100	100	98	100	100	96	86	82	7
Muck	96	100	100	100	100	100	98	96	85

higher residual mortality in the muck than in either of the loam soils. Forty-one days after treatment Isolan in clay loam, silt loam and muck gave 47, 7, and 85% mortality, respectively.

Phytotoxicity.—Phytotoxic effects occurred from all insecticides because of the high rates used. The apparent degree of phytotoxicity caused by the five insecticides and/or their metabolites 15 days after treatment was as follows: Isolan > Thimet > Phosdrin = schradan > demeton. Isolan and Thimet produced the earliest effects, which were evident 5 days after treatment. The xylene, used as a solvent with all of the insecticides caused a slight, marginal wilting and necrosis on control plants grown in quartz sand.

The damage from Isolan consisted of progressive wilting, chlorosis, and finally, death of the leaves. At first, only the outer margins were affected, but eventually the whole leaf became necrotic. The lowest leaves were damaged more severely than the others. As was the case with all insecticides, very little additional phytotoxicity resulted after 2 to 3 weeks. The degree of phytotoxicity caused by Isolan in the various soils occurred in the following order: quartz sand > beach sand > Plainfield sand > clay loam = muck. No injury was observed on peas grown in the silt loam.

The symptoms of Thimet toxicity were first evident on plants grown in quartz sand and beach sand as individual spots on all foliage except the growing point. Fifteen days after treatment damage appeared as light colored, circular, necrotic indentations with reddish-purple borders which tended to be concentrated at the outer margins of the leaves. In extreme cases the whole edge of the leaf became necrotic and curled inward. After 25 days the new growth was no longer affected. Plants grown in muck and silt loam were not visibly affected at any time during the test. Thimet in quartz sand caused the most damage followed by the toxicant in beach sand, Plainfield sand, and clay loam.

The damage resulting from schradan and Phosdrin was almost identical. The lower leaves became wilted, necrotic, and bleached to a grayish-white at their outer margins. An inward curling eventually resulted on most of the damaged leaves. The damaged areas caused by schradan had definite margins while the damage from Phosdrin gradually blended into the healthy tissue. Symptoms from both insecticides appeared on peas in quartz sand and beach sand.

Demeton caused the least apparent phytotoxicity but also the most peculiar. Three types of damage were visible 15 days after treatment. The first, evident on all soils except muck was observed 7 days after treatment. The extreme outward margins of many of the leaves and stipules, especially on the upper two-thirds of the plant, were curled slightly upward. No necrosis or spotting was visible on the curled leaves. The second type of damage, present on the extreme lower foliage, consisted of marginal, downward-curling, necrotic areas at the apical end of the stipules on plants grown in quartz sand. The third type of phytotoxicity caused by demeton, and located on leaf margins, consisted of reddish-bordered spots similar to those caused by Thimet only much smaller. These spots were evident on plants grown in beach and quartz sand. It is possible that the three types of damage caused from applications of demeton may have resulted from metabolites and/or impurities.

Cholinesterase Assay.—Pea plants grown in the six soil materials and treated with Isolan and Phosdrin in a manner similar to that described for bioassay were used for measuring cholinesterase activity. Treated plants were cut off at the soil surface at various intervals after treatment (1.5, 3, 6, and 12 hours, and 1, 2, 4, 7, and 14 days for Phosdrin and 2, 6, 18, and 54 hours, and 7, 14, 21, and 28 days for Isolan) and frozen for later anticholinesterase assay. Duplicate cholinesterase analyses were made on 5-gram samples from each treatment based on the procedure described by Hensel *et al.* (1954) and modified by Casida *et al.* (1956). A homogenate of the pea tissue was prepared by macerating 5 grams of frozen tissue in an equal weight of water with a mortar and pestle and straining the brei through four thicknesses of cheesecloth. Serial dilutions of the homogenate adjusted to pH 8.0 were added to 3 ml. of whole human blood at pH 8.0 in centrifuge tubes and brought to a total volume of 10 ml. with distilled water. After incubation at 37.5° C. for 60 minutes, 1 ml. from each tube was added to 1 ml. of a phosphate buffer (pH 8.0) in small beakers. Following the initial pH readings, 0.2 ml. of acetylcholine was added to each beaker, after which the samples were incubated at 37.5° C. for 2 hours. The changes in pH were then determined and the results compared with standard curves prepared by adding known amounts of the technical insecticide to untreated pea foliage and analyzed by the same procedure. The results, expressed as p.p.m., are found in tables 3 and 4.

Phosdrin was absorbed and translocated very rapidly. The toxicant could be detected in varying amounts in the plants from all six soil materials 1.5 hours after treatment. Plants grown in quartz sand absorbed the insecticide in the largest amounts with the highest concentration (170 p.p.m.) occurring 2 days after treatment. Fourteen days after treatment the plants in quartz sand still contained 8.5 p.p.m. of insecticide. Greater absorption of Phosdrin occurrred from beach sand than from agricultural soils, but much less than from quartz sand. The

Table 3.—Phosdrin residues in peas following treatment to various soils (anticholinesterase assay).

Soil	P.P.M. Residue After Time Indicated								
	1.5 Hrs.	3 Hrs.	6 Hrs.	12 Hrs.	1 Day	2 Days	4 Days	7 Days	14 Days
Quartz sand	3.4	11.0	12.5	30.6	79.0	170.0	81.7	24.0	8.50
Beach sand	4.4	10.5	11.8	18.4	12.8	13.3	2.80	0.41	0.33
Plainfield sand	0.63	1.04	1.20	2.0	0.53	0.21	<.05	—	—
Superior clay loam	0.52	1.20	1.01	0.63	0.40	0.14	<.05	—	—
Clyde silt loam	0.49	0.68	0.92	1.21	0.43	<.05	—	—	—
Muck	0.46	0.77	0.82	0.43	0.25	<.05	—	—	—

Table 4.—Isolan residues in peas following treatment to various soils (anticholinesterase assay).

Soil	P.P.M. Residue After Time Indicated							
	2 Hrs.	6 Hrs.	18 Hrs.	54 Hrs.	7 Days	14 Days	21 Days	28 Days
Quartz sand	0.72	6.5	45.8	310.0	437.0	476.0	564.0	526.0
Beach sand	1.00	6.5	32.4	180.0	437.0	287.0	314.0	263.0
Plainfield sand	<.10	0.72	6.2	52.0	124.0	121.0	116.0	159.0
Superior clay loam	<.10	3.60	18.0	37.5	56.8	46.2	23.7	17.0
Clyde silt loam	<.10	<.10	3.6	28.4	37.5	30.7	15.9	7.8
Muck	<.10	2.50	2.7	14.1	30.1	62.5	30.1	40.4

small amounts of toxicant found 7 and 14 days after treatment in plants from beach sand are probably present because slight amounts of the insecticide are made available to the plant roots from time to time.

The four agricultural soils prevented pea plants from absorbing any great amounts of Phosdrin. Maximum insecticide concentrations in the plants occurred 3 to 12 hours after treatment. A slightly greater uptake was evident with plants grown in the Plainfield sand than in the three heavier soils. Very little toxicant could be detected 4 days after treatment in plants grown in the Plainfield sand and Superior clay loam, and 2 days after treatment for plants grown in Clyde silt loam and muck.

The extreme phytotoxic effects of Isolan occurring on plants grown in the quartz, beach, and Plainfield sand caused a great deal of necrosis on a large portion of all plants which resulted in a loss of the total fresh weight. Since the results are based on fresh weight, the values are approximately 30 to 40% too great for the 14, 21, and 28 day samples taken from the quartz and sands, and 10 to 20% too great for samples taken from the Plainfield sand.

The absorption of Isolan by peas was much slower than that of Phosdrin but the former was detected for a greater length of time than the latter in plants grown in all soil materials (tables 3 and 4). Absorption of Isolan from the three sands was much greater than that from the three heavier soils. The highest Isolan concentrations were found in peas grown in quartz followed by plants grown in beach and Plainfield sand with maximum toxicant concentrations occurring after 54 hours following treatment. The peas in the Superior clay loam absorbed more toxicant initially than those grown in the Plainfield sand. However, after 54 hours insecticide concentrations in plant tissue from the Plainfield sand increased sharply. Less Isolan was absorbed from the silt loam than from the other soils. Although the insecticide concentrations in peas grown in muck were lower than those of plants grown in the two loam soils during the first 7 days, analysis at 14, 21, and 28 days indicated higher concentrations of Isolan in plants from muck. Peas grown in the two loam soils contained the maximum concentration of Isolan 7 days after treatment while peas in muck contained the highest concentrations 14 days after treatment.

INSECTICIDE BINDING IN SOILS.—Thirteen soils were collected from different areas of Wisconsin to obtain a representative sample of the soil types in the State. The ability of each soil to bind radioactive Phosdrin was determined by the following procedure.

Five inches of the soil to be tested were placed in a small column chromatographic tube (7/16 inches diameter). Enough distilled water was added to completely saturate the soil column. Then 0.25 mg. of radioactive Phosdrin (prepared by the method described by Casida *et al.* 1956) plus 0.14 mg. of nonradioactive Phosdrin in 5 ml. of water was added to the top of the soil column. With the aid of pressure, and two 2-ml. portions of water, the radioactive solution was forced beneath the soil surface. Distilled water was then forced through the soil with the aid of pressure (2 to 15 lbs. per sq. in. depending upon the soil type) and collected in 1-ml. aliquots until radioactivity could no longer be detected in the eluate. By analyzing the individual aliquots the rate of leaching through the different soils could be determined. Two 3-gram samples of moist soil were taken from the column (one from the bottom half and one from the top half), placed in planchets, air dried for 24 hours and weighed. The amount of bound radioactive Phosdrin was determined with the aid of a Geiger-Mueller Counter from the air-dried soil. The radioactivity adsorbed by the soils was determined, corrections made for decay and results recorded as micrograms of Phosdrin bound per 100 grams of soil. Results were taken from two replicates of each soil.

Samples of the 13 soils were sent to the Soils Department of the University of Wisconsin and analyzed for organic matter; base exchange capacity; pH; pounds per acre of nitrogen, potassium and phosphorus; and percentage of sand, silt and clay. A list of the soils and their various characteristics plus their ability to bind Phosdrin is found in table 5.

An experiment designed to obtain data similar to that described above was conducted on three soils and a pure clay with radioactive phorate (preparation procedure described by Bowman & Casida 1957). Because of the difficulty encountered in leaching water through a column of muck soil or pure clay, a procedure differing from that described for Phosdrin was used. Ten micrograms of labelled phorate in 5 ml. of water were added to 2 cubic centimeters of the soil or clay in a test tube. The tubes were then shaken for 5 minutes and centrifuged at 20,000 r.p.m. until the liquid became free of suspended materials. The decantant liquid was discarded and after adding 10 ml. of distilled water, the tubes were again shaken and centrifuged. The samples were washed in this manner until no radioactivity could be detected in the decantate (usually 7 to 10 times). After air-drying the materials for 24 hours 1-gram samples were counted. The results obtained from an average of three replications are expressed as the micrograms of phorate bound per 100 grams of the material (table 5).

The rate of Phosdrin leaching was determined for the 12 soil types used in the experiment by counting the leachate in 1-ml. aliquots. Because all of the mineral soils

Table 5.—Amounts of Phosdrin and phorate bound by various soils and some physical and chemical characteristics possibly responsible for insecticide retention.

Soil	Bound Insecticide[a]	Characteristics of Soils								
		Organic Matter (%)	Base Exchange Capacity[b]	pH	Lb. per A. of			Mechanical Composition (%)		
					N	P	K	Sand	Silt	Clay
	Phosdrin									
Peat	127.0	70.0	120.0	5.9	200	10	107	75	20	5
Spencer silt loam	27.1	3.4	26.0	5.3	350	20	400	40	46	14
Carrington silt loam	18.1	3.4	24.7	7.0	250	30	56	20	60	20
Miami gravelly loam	16.8	3.2	12.5	6.5	250	90	363	50	36	14
Fox silt loam	15.8	3.0	12.8	8.0	175	100	130	30	63	7
Miami silt loam	13.8	2.0	14.4	8.0	175	170	116	63	30	7
Clyde clay loam	12.0	3.6	21.8	5.4	250	150	600	30	40	30
Antigo silt loam	9.9	3.2	10.0	5.2	225	110	242	20	73	7
Hancock sand	8.1	0.5	3.5	6.5	100	80	144	75	15	10
Kennan sandy loam	3.8	0.4	3.6	7.0	50	20	56	50	38	12
Plainfield sand	3.4	0.8	4.3	5.3	100	190	307	80	10	10
Hiawatha sandy loam	3.1	0.2	3.1	8.0	50	5	47	68	25	7
	Phorate									
Muck	280.0	54.0	65.0	6.2	800	300	1050	78	11	11
Clyde silt loam	42.0	3.6	32.3	6.8	400	400	930	4	76	20
Plainfield sand	6.4	0.8	4.3	5.3	175	170	145	83	8	9
Kaolinite (pure clay)	6.0	0.0	35.0	—	—	—	—	0	0	100

[a] Values indicate micrograms of insecticide bound by 100 grams of soil.
[b] Base exchange capacity expressed as milliequivalents per 100 grams of soil.

showed similar rates of leaching the data from only three representative types are shown (fig. 1). A similar leaching rate occurred in the sandy soils and heavier loam soils with a slightly faster elution of Phosdrin occurring from the former. An extremely slow rate of insecticide leaching resulted in the high organic matter-containing peat soil. The radioactivity was eluted in sharp peaks from the mineral soils, while in the peat the toxicant was leached very slowly and distributed over a large volume of leachate.

Relatively small quantities of Phosdrin were bound by the 12 soils tested with amounts varying from 127 micrograms per 100 grams in the peat to 3.1 micrograms per 100 grams in the Hiawatha sandy loam (table 5). On a weight basis, as used in this test, a great deal more adsorption occurred in the peat than in any other soil. On a volume basis, which would more closely approximate field conditions, the amount of Phosdrin adsorbed by the peat was comparable to that bound by the Spencer silt loam. The clay and silt loam soils tended to bind more Phosdrin than the sandy loams and sands. Phorate was adsorbed in the greatest amounts by the muck soil, followed by the Clyde silt loam, and finally by the Plainfield sand and kaolinite where relatively little binding occurred.

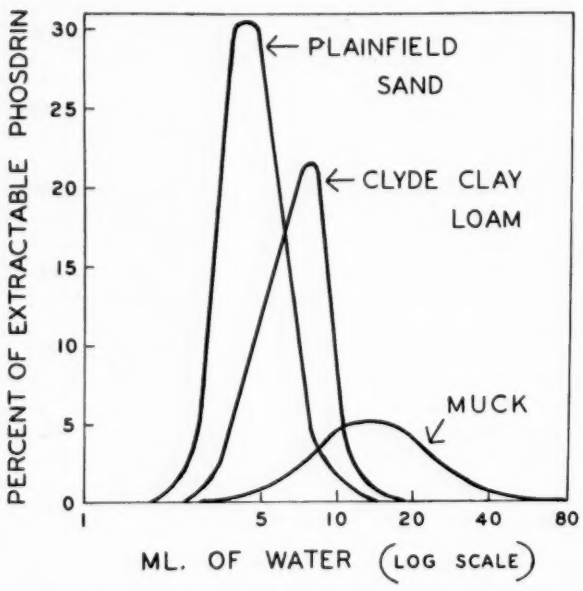

FIG. 1.—The rate at which Phosdrin leached through columns of three soils as indicated by the per cent of extractable radioactivity found in the leachate.

As a general rule, soils with a greater base exchange capacity (B. E. C.) and a higher organic matter content adsorbed more insecticide than soils with a low base exchange capacity and a low organic matter content. Base exchange refers to an exchange of bases between two salts or compounds that are in contact under such conditions that at least one of them is in solution so that the exchange reaction may take place (Truog *et al.* 1947). Both the inorganic and organic portions of the soil contribute to base exchange. In the case of the inorganic portion it is the amount and type of clay minerals that are largely responsible for this property. The humus provides the base exchange capacity in the organic portion. Statistical analyses (table 6) indicated that the amount of Phosdrin bound by the various soils correlated to a high degree with organic matter content ($r = 0.931$) and base exchange capacity ($r = 0.928$), and to a lesser degree with available nitrogen ($r = 0.655$). The mechanical composition, pH, and available phosphorus and potassium do not correlate with Phosdrin binding. It is very unlikely that Phosdrin binding is affected directly by base exchange mechanisms because the toxicant does not ionize and is relatively nonpolar. Also, a pure clay (kaolinite) which had a base exchange capacity of 35.0 milliequivalents per

Table 6.—Coefficients of correlation between the amount of Phosdrin bound by 12 soils and their various physical and chemical characteristics.

Soil Characteristics[a]	Coefficients of Correlation Between Bound Phosdrin and Soil Characteristics[b]
Organic matter content	0.931**
Base exchange capacity	0.928**
Soil pH	−0.020 n.s.
Available nitrogen content	0.655*
Available phosphorus content	−0.260 n.s.
Available potassium content	0.070 n.s.
Mechanical composition (%)	
sand	−0.140 n.s.
silt	0.080 n.s.
clay	−0.030 n.s.

[a] See table 5 for data.
[b] Bound Phosdrin values and base exchange values were transformed to logarithms for analysis. Organic matter values were first multiplied by 10 and then changed to logarithms. The remaining values were analyzed as shown.

100 grams of soil did not bind phorate in any great amount. Since base exchange is also very dependent upon organic matter content it is likely that organic matter or some factor associated with it provides the direct mechanism for insecticide binding in soils. The effect of nitrogen upon insecticide binding has not been studied but the correlation between the two is probably an indirect association since loam and clay soils, and high organic-containing soils tend to contain greater amounts of available nitrogen than sandy soils or low organic-containing soils.

Compared with insecticides such as phorate, Isolan and demeton, Phosdrin is a very inefficient systemic insecticide for use in soil as indicated by pea aphid bioassay and anticholinesterase analysis. However, the data presented here on the binding of Phosdrin by soil does give an indication of what might be expected with most organophosphorus insecticides.

DISCUSSION AND CONCLUSIONS.—Plants were able to absorb large amounts of insecticide from sands that were relatively free of organic matter as indicated by bioassay, cholinesterase analysis and phytotoxicity. Only a small amount of the insecticide added to an agricultural soil was absorbed by the plant, but this type of application provided long residual effectiveness with certain toxicants because they were applied in high concentrations. A soil application of Phosdrin gave extremely short residual effectiveness. A combination of soil adsorption plus rapid hydrolysis in, and volatilization from the plant resulted in very low residue concentrations. Even high rates of Phosdrin, such as used in these experiments, lasted no longer than 3 days in heavier soils as compared with 40 days or more for other insecticides applied at the same rate. In sands Phosdrin gave longer residual kill than in agricultural soils, apparently because considerably more of the toxicant was available for plant absorption. In these tests schradan was relatively ineffective as a soil treatment. Insect mortality was based on 24-hour readings and this appears to be too short a time for schradan to act. Phorate, demeton and Isolan were very effective in controlling insects when applied to both sands and agricultural soils. These compounds persisted in the soil and plants for long periods of time, and thus provided an excellent reservoir of insecticide. With the exception of Isolan, all the insecticides were absorbed in larger amounts from the loam soils than from the muck soils. However, anticholinesterase, bioassay and phytotoxicity data indicated that Isolan was more effective in muck than in either clay loam or silt loam. Isolan differs from the other four systemic toxicants used in that it does not contain a phosphorus atom in the molecule. It is possible that Isolan is not adsorbed by organic matter to such a degree as are the other insecticides, or it is also possible that Isolan is bound by the higher amounts of clay materials found in the clay and silt loam. Either of these two explanations could account for the difference between the uptake of Isolan and the other compounds by pea plants from the treated soils.

Phosdrin was bound in small amounts to the 12 soils tested against the leaching action of water, with a greater degree of binding occurring in soils with a high base exchange capacity and a high organic matter content. Since phorate was not bound to any extent by a pure clay and since base exchange capacity is partially dependent upon the organic matter, it is likely that the organic matter or some portion of it has the most effect on insecticide binding.

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Effects of Moisture on the Clover Root Borer and Red Clover Yields[1]

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Abstract

Rainfall and populations of the clover root borer, *Hylastinus obscurus* (Marsham), were artificially controlled in pots of red clover grown under shelters in the field. Populations of clover root borer were higher under dry conditions. Although yield losses were high in borer-infested pots, yield reductions were no greater under dry than under wet conditions.

It is generally believed that the clover root borer, *Hylastinus obscurus* (Marsham), causes more damage in dry than wet seasons (Riley 1879, Folsom 1909, Rockwood 1926). Rockwood (1926) also thought development was more rapid and populations higher under dry conditions. Little experimental evidence is available to confirm these observations.

Procedure.—To test these observations more accurately, red clover was grown under artificially controlled moisture conditions. Two shelters, each 40 feet long, 2 feet wide, and 18 inches high on the sides, were covered with gable-type celloglass roofs. Open sides on these shelters permitted free wind movement. The celloglass roof allowed light penetration while shedding most rainfall. Glazed tile pots, 9 inches in diameter, were buried to their rims under these shelters and filled with a mixture of sand and compost. Kenland red clover plants dug from a nearby field were reset in these pots. First-harvest-year plants were potted on April 15 for the 1955 test and seedling plants on September 12, 1955, for the 1956 test. Two plants per pot were used in 1955 and four in 1956. The shelters were roofed and artificial watering begun during April of both years.

Four moisture levels were used, each level with and without clover root borers. Treatments were applied in a randomized block replicated 10 times. Half the pots were treated with dieldrin granules at 2 pounds actual per acre to prevent root borer infestation. Ten adult root borers were released in each untreated pot prior to spring migration and additional dead infested roots containing an unknown number of borers were placed in each pot to permit normal seasonal emergence of the adults. During the 2 years, only one plant became infested in all the dieldrin-treated pots.

Plants were watered twice weekly. Rates used in 1955 were equivalent to 1, 2, 4, and 6 inches of rainfall per month. It was found in 1955 that 6 inches was but little better for clover growth than 4 inches and that only 1 inch still permitted fair growth. Therefore 1956 rates were changed to ½, 1, 2, and 4 inches per month. Unfortunately driving rains blew under the shelters in 1956, obscuring moisture effects during the first two cuttings. No water was added to any pots between June 15 and July 17 and only to the higher levels after this period in a rather unsuccessful attempt to reestablish a moisture differential.

Three cuttings of hay were made each year; June 2, July 18, and August 25, 1955, and June 12, July 24, and September 12, 1956. Both green and dry yields were recorded for each pot. Immediately after the third cutting, roots were dug and dissected to determine the number and stage of root borers present.

Table 1.—Analyses of variance of yield data.

Factor	Degrees Freedom	1955 Test Mean Square	1955 Test F	1956 Test Mean Square	1956 Test F
Water	3	1,919.7	47.40[a]	61.3	0.78
Borers	1	3,353.0	82.79[a]	2,136.0	27.28[a]
Cutting	2	11,400.5	281.49[a]	16,950.5	216.48[a]
W×B	3	25.3	0.62	161.0	2.06
W×C	6	339.0	8.37[a]	118.0	1.51
B×C	2	593.5	14.65[a]	135.0	1.72
W×B×C	6	16.5	0.41	280.5	3.58[a]
Error	207	40.5		78.3	

[a] Significant at 1% level.

Results.—Analyses of green and dry yields gave almost identical results. Therefore only dry yield data are reported here. The analyses of variance are given in table 1 and yield data in table 2.

In the 1955 test, root borers significantly reduced yields in all three cuttings with the per cent loss being greatest in the third cutting and least in the first. Yields were increased as water was increased in the first two cuttings but water had little effect in the third cutting. The interaction between borers and water was not significant, indicating that moisture had little, if any, effect on root borer damage.

Results for the 1956 test were similar to those in 1955 except that moisture effects were largely obscured by rain blowing under the shelters. Borer-infested pots yielded less during all three cuttings, with highest losses again occurring in the third cutting. In 1956, as in 1955, no interaction occurred between borers and moisture. The significant second order interaction between water, borers, and cutting probably reflects the decreased yields in borer-infested pots in the three high-water levels relative to the low level during the first two cuttings. Since a true moisture differential was not maintained during the first two cuttings, no explanation of this effect is available.

In general, losses were about the same regardless of soil moisture. It appears that while moisture has little effect on clover root borer damage, such damage has been most often noticed during dry seasons when the second cutting of red clover hay was small. During such seasons plants are most often examined for insects and, because of its presence, the clover root borer likely received much of the blame actually caused by climatic conditions.

Yield reductions were much greater during both seasons than would be expected from the low populations of

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Table 2.—Dry yield in grams for uninfested pots and per cent yield reduction attributed to clover root borer.

Cutting		Inches Water Per Month					Mean
		0.5	1	2	4	6	
				1955			
1	Y[a]		11.5	16.6	26.0	29.0	20.8
	R[b]		21.8	22.3	22.7	9.7	18.0
2	Y		24.9	37.5	43.1	45.2	37.7
	R		39.8	44.8	30.9	33.8	36.4
3	Y		7.4	9.4	10.6	10.3	9.4
	R		58.1	52.1	50.0	52.4	52.8
Mean	Y		14.6	21.2	26.6	28.2	
	R		38.1	40.0	30.2	27.8	33.0
				1956			
1	Y	40.1	39.7	40.9	35.8		39.1
	R	9.3	15.1	34.2	22.1		19.6
2	Y	26.6	28.4	28.7	28.2		28.0
	R	3.8	32.7	40.4	25.5		26.0
3	Y	5.2	8.8	6.7	11.1		8.0
	R	13.5	59.1	34.3	33.3		37.4
Mean	Y	24.0	25.6	25.4	25.0		
	R	6.1	26.7	36.6	25.0		23.9

[a] Mean yield of uninfested pots.
[b] Per cent reduction in yield attributed to borers.

clover root borers present (Pruess & Weaver 1958). It is possible that other factors obscured the true root borer effects. Meadow spittlebugs, *Philaenus leucophthalmus* (L.), were present in 1955 and were handpicked. The cornfield ant, *Lasius alienus americanus* Emery, became abundant during the summer of 1955 and a brown-sugar-Paris-green bait was used but proved rather ineffective. Only low populations of aphids and tarnished plant bugs, *Lygus lineolaris* (P. de B.), were observed in 1956 and no controls were used. Mealybugs were abundant on the roots in untreated pots during both seasons.

Clover root borer populations were highest in the low moisture levels and lowest at the high levels (table 3). Similar findings were reported by Koehler & Gyrisco (1959). Root borer counts were transformed by the inverse hyperbolic sine transformation before analysing. The inverse hyperbolic sine transformation, given by $x' = q^{-1/2}\sinh^{-1}(qx)^{1/2}$, appears appropriate for clover root borer data (Pruess & Weaver 1959). Differences among treatment means were just significant at the 5% level.

Table 3.—Mean number of clover root borers per pot in different moisture treatments.

Year	Inches Water Per Month				
	0.5	1	2	4	6
1955		5.0	2.2	3.3	1.5
1956	4.6	5.7	3.8	1.9	
Mean	4.6	5.4	3.0	2.6	1.5

An analysis of regression of root borers on water gave a highly significant regression coefficient of $b = -0.659$. It has been observed that number of borers per root tends to increase as root size increases (Newsom 1948, Pruess & Weaver 1958). However, measurements of crown diameters showed no differences in root size to exist among moisture treatments. This then could not explain differences in root borer populations in the moisture treatments. Koehler and Gyrisco (1959), however, believed that under field conditions, root size had a greater influence on borer numbers than soil moisture. If clover root borer populations are higher during dry seasons, as field observations made in Ohio have indicated, these larger populations may account for reduced yields. This appears to be a more logical explanation for reduced yields in dry seasons than a direct interaction between borers and moisture. Since nearly all borers were adults at the time roots were dug, no differences in stage of development owing to soil moisture could be detected in these tests.

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Relative Toxicity, Knockdown Effectiveness, and Intensity of Synergism of Mixtures of Piperonyl Butoxide with Barthrin and Some of Its Isomers[1]

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Abstract

When applied to house flies, *Musca domestica* L., in 10:1 proportion as space sprays on the Campbell turntable, mixtures of piperonyl butoxide with the 6-chloropiperonyl esters of *d-trans-*, *dl-trans-*, *dl-cis-*, and *dl-cis-trans-*(barthrin) chrysanthemumic acid were, respectively, 1.72, 1.12, 0.843, and 1.06 as toxic and 0.246, 0.172, 0.141, and 0.166 as effective in knockdown as allethrin alone. Synergism with respect to both lethality and knockdown was demonstrated in all mixtures, the intensity ratios being about 3.0. The range for relative toxicity between 5 and 95% mortality levels was from 69 to 146% of the toxicity at the 50% level, and the range for intensity of synergism was 94 to 107% of the intensity at that level.

With the synthesis of barthrin, the 6-chloropiperonyl ester of *dl-cis-trans*-chrysanthemumic acid (Barthel & Alexander 1958), and the estimation of its relatively high toxicity to house flies, *Musca domestica* L. (Gersdorff & Piquett 1959), there arose the question of its synergizability. The preparation of some of the barthrin isomers and measurement of their relative toxicity interested the authors in this question. Tests to study the joint action of mixtures of piperonyl butoxide with the esters and to measure the intensity of any synergistic action demonstrated are reported in this paper.

Materials.—The esters studied were barthrin and its *d-trans*, *dl-trans*, and *dl-cis* isomers. Their preparation and description were given and their relative toxicities to the house fly were determined by Gersdorff *et al.* (1959).

The sample of piperonyl butoxide was the same as that used in previous studies with other pyrethroids (Gersdorff & Piquett 1958).

A sample of allethrin analyzing 93.4% by the hydrogenolysis method was used as the principal standard of comparison. As an additional standard a sample of pyrethrins was used. It was the unrefined extractive in which 54.2% of the total pyrethrins consisted of pyrethrin I and cinerin I as determined by the mercury-reduction method.

Sprays were prepared by dissolving the materials in refined kerosene (Deobase) and, after preliminary tests, were diluted to selected concentrations with the same solvent. The sprays containing piperonyl butoxide were prepared in the proportion of 10 times as much synergist as pyrethroid.

Knockdown and Mortality.—These sprays were applied by the Campbell turntable method to house flies of the National Association of Insecticide and Disinfectant Manufacturers' 1948 strain. All sprays were tested simultaneously; once on each of six populations. About 111 flies, averaging 2 to 3 days in age, were used in each test. Table 1 summarizes the knockdown and mortality data.

Evaluation of Relative Toxicity and Intensity of Synergism.—To evaluate relative toxicity and intensity of synergism and to determine the precision of the estimates, the mortality data were subjected to probit analysis essentially as described by Finney (1952).

In a study of provisional regression lines it appeared that the data for the piperonyl butoxide mixtures should be fitted with parallel lines of steeper slope than those for the standards. Therefore, a mean slope was calculated for the mixtures and equations were determined for lines of this slope. The individual provisional equations were determined for the standards. Expected probits were computed from the equations, and the statistical procedures were followed. Analysis of chi-squares subsequent to generalization gave no evidence of conflict with the hypothesis regarding parallelism of lines for the mixtures. A heterogeneity factor, 5.523, was required in the calculation of error estimates for the mixtures, 8.114 for allethrin, and

Table 1.—Mortality and knockdown of house flies caused by kerosene sprays selected to study the comparative effect of piperonyl butoxide in mixtures with barthrin and some of its isomers.[a]

Material	Concentration (Mg. per Dl.)			Per Cent	
	Pyrethroid	Piperonyl Butoxide	Pyrethroid Equivalents[b]	Knockdown in 25 Minutes	Mortality in 1 Day
Piperonyl butoxide plus—					
d-trans isomer	145.8	1458	170.8	98.7	99.2
	102.0	1020	119.5	92.5	86.2
	71.43	714.3	83.67	64.1	65.2
	50.00	500.0	58.57	49.2	26.0
	35.00	350.0	41.00	21.7	9.3
	24.50	245.0	28.70	5.1	2.1
dl-trans isomer	204.1	2041	261.2	98.0	97.8
	142.9	1429	182.9	82.9	90.8
	100.0	1000	128.0	74.6	55.9
	70.00	700.0	89.59	46.5	30.0
	49.0	490.0	62.72	18.4	11.2
dl-cis isomer	204.1	2041	277.7	96.2	93.2
	142.9	1429	194.4	81.6	72.4
	100.0	1000	136.1	51.4	43.0
	70.00	700	95.24	29.2	15.7
	49.00	490.0	66.66	11.0	2.2
barthrin	204.1	2041	271.3	96.5	97.7
	142.9	1429	189.9	82.7	82.6
	100.0	1000	132.9	62.3	52.9
	70.0	700	93.04	45.7	33.5
	49.00	490.0	65.13	23.6	14.2
Allethrin	214.1	—	—	100	75.1
	142.8	—	—	100	64.9
	95.17	—	—	100	33.6
	63.45	—	—	100	11.1
	42.30	—	—	99.7	6.6
	28.20	—	—	97.0	1.6
Pyrethrins	759.4	—	—	100	81.1
	506.3	—	—	100	69.8
	337.5	—	—	100	57.4
	225.0	—	—	100	34.4
	150.0	—	—	100	15.0
	100.0	—	—	100	8.6

[a] Six replicates, about 111 flies each.

[b] The pyrethroid equivalents of piperonyl butoxide are 0.01714 for the *d-trans*, 0.02799 for the *dl-trans*, 0.03605 for the *dl-cis* isomer, and 0.03292 for barthrin.

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4.414 for pyrethrins. The standard errors of the final regression coefficients were 0.173 for the mixtures, 0.284 for allethrin, and 0.169 for pyrethrins.

Given below are the final equations showing the regression of mortality, expressed in probits, on concentration in milligrams per deciliter expressed as logarithms. Those for the piperonyl butoxide mixtures are given on two bases, the expression of concentration in terms of pyrethroid content only (with constant 1) and of pyrethroid equivalents (with constant 2).

	(1)	(2)
Piperonyl butoxide plus—		
d-trans isomer	$Y=5.2005X-4.3312$	-4.6886
dl-trans isomer	$Y=5.2005X-5.0930$	-5.6503
dl-cis isomer	$Y=5.2005X-5.5997$	-6.2950
barthrin	$Y=5.2005X-5.1321$	-5.7748
Allethrin	$Y=3.4308X-2.1975$	—
Pyrethrins	$Y=2.6781X-1.7285$	—

From these equations the median lethal concentrations (LC-50's) were estimated and relative toxicities were calculated as the inverse ratios of the relevant pair of estimations on the pyrethroid equivalent basis. They are given in table 2. Each pyrethroid equivalent of piperonyl butoxide was calculated by means of the toxicities relative to allethrin of piperonyl butoxide (Gersdorff *et al.* 1957) and the pyrethroids (Gersdorff *et al.* 1959). These equivalents are given in table 1.

The ratio of the toxicity of a mixture to that of the pyrethroid alone will, when significantly greater than unity, furnish an estimate of the intensity of synergism. The ratios and their standard errors are given in table 2. Also given are logarithms of the intensity ratios and the minimum logarithms required to demonstrate synergism. Since the former easily exceed the latter, synergism is demonstrated for all piperonyl butoxide mixtures.

DISCUSSION OF TOXIC EFFECTS.—From table 2 it may be calculated that allethrin is 2.60 as toxic as pyrethrins. Multiplication of the toxicities of the mixtures relative to allethrin by this ratio will give estimates of the toxicities relative to pyrethrins. Thus, it may be stated that the piperonyl butoxide mixtures of the *d-trans* isomer, the *dl-trans* isomer, barthrin, and the *dl-cis* isomer were, respectively, 1.72, 1.12, 1.06, and 0.84 as toxic as allethrin and 4.46, 2.91, 2.76, and 2.19 as toxic as pyrethrins.

It is apparent from table 2 that the synergism intensity at the 50% mortality level is threefold for barthrin and each of the isomers. We deduced that this ratio would also apply to the *l-trans* isomer under similar conditions, but no deduction can be made regarding the ratios for the optically active *cis* isomers. However, it is unlikely that they would differ greatly. The ratios found for those isomers tested are practically the same as for allethrin and its isomers in similar piperonyl butoxide mixtures (Gersdorff & Piquett 1958).

A corollary of finding similar intensities is that the four mixtures possess about the same toxicity relative to each other on the equivalent basis as do the esters alone. The estimated ratios for barthrin, the *dl-trans* isomer, and the *d-trans* isomer in comparison with the *dl-cis* isomer as unity are, respectively, when synergized 1.26, 1.33, and 2.04 and when alone 1.10, 1.29, and 2.10.

Because of differences in slope of regression lines, the estimates for relative toxicity and intensity of synergism will be different at different mortality levels. Allethrin has a lower slope than barthrin and the isomers (Gersdorff *et al.* 1959). Therefore, the estimates of relative toxicity, based on allethrin, will differ more widely than will the estimates for synergism intensity. LC-5's and LC-95's, were determined directly from the equations based on equivalents, and ratios for relative toxicity and intensity of synergism were estimated. The latter are given in table 2 to indicate the approximate ranges possible; they are in fact so narrow as to be within the limits of tolerance of the intensity at the 50% level. With all four mixtures the range for relative toxicity is 69 to 146% of the toxicity at the 50% mortality level and the range for intensity of synergism is 94 to 107% of the intensity at that level.

Expanding the picture for ready comparison, LC-50's for allethrin in a similar synergistic mixture and for barthrin alone against these fly populations were calculated by methods that are permissible because ratios of toxicity obtained by this testing procedure are reproducible. The intensity of synergism (which is a ratio of toxicity) for allethrin under like conditions was demonstrated in six extensive studies to be 2.63 to 3.12, with a mean of 2.960 (Gersdorff & Piquett 1958). From the latter figure and the LC-50 for the allethrin standard in this study we may deduce that the LC-50 for a similarly synergized allethrin mixture against these flies would be 42.33 mg. per deciliter (125.3 divided by 2.960). The mean

Table 2.—Evaluation of the relative effects on toxicity of piperonyl butoxide in mixtures with barthrin and some of its isomers.

MATERIAL	LC-50 (MG. PER DL.) Original Basis[a]	Pyrethroid Equivalent Basis	Allethrin Equivalent Basis	RELATIVE STANDARD ERROR OF ORIGINAL LC-50 (%)	RELATIVE TOXICITY OF: Mixture on Pyrethroid Equivalent Basis	Principal Toxicant Alone[b]	INTENSITY OF SYNERGISM AT 50% MORTALITY LEVEL[c]	LOG OF INTENSITY RATIO	Minimum LOG REQUIRED TO DEMONSTRATE SYNERGISM	RANGE OF INTENSITY BETWEEN 5 AND 95% LEVELS
Piperonyl butoxide plus—										
d-trans isomer	62.27+ 622.7	72.94	45.12	2.78	1.718	0.6186	2.777±0.106	0.4436	0.0326	2.60–2.97
dl-trans isomer	87.25+ 872.5	111.7	42.29	2.83	1.122	.3787	2.963±0.111	.4717	.0320	2.77–3.16
dl-cis isomer	109.20+1092.0	148.6	43.68	2.82	0.8434	.2940	2.869±0.104	.4577	.0308	2.69–3.06
barthrin	88.77+ 887.7	118.0	37.99	2.73	1.062	.3220	3.298±0.118	.5183	.0304	3.09–3.52
allethrin[d]	38.27+ 382.7	42.33	42.33	—	2.960	1.0	2.960	—	—	—
Barthrin[d]	399.1	399.1	125.3	—	—	0.3126	—	—	—	—
Allethrin	125.3	125.3	125.3	4.95	1.0	1.0	—	—	—	—
Pyrethrins	325.4	325.4	125.3	4.05	—	0.3850	—	—	—	—

[a] For the compound expressions the first figure refers to the pyrethroid.
[b] Gersdorff *et al.* 1959.
[c] Minimum ratio required to indicate synergism is 1.08.
[d] Calculated means as described in text.

Table 3.—Evaluation of relative effects on paralytic action of piperonyl butoxide in mixtures with barthrin and some of its isomers.

Material	DC-50 (Mg. per Dl.)		Relative Knockdown Effectiveness of—		Ratio of Joint Effectiveness to Expected	Ratio of Relative Knockdown Effectiveness to Relative Toxicity[b]
	Pyrethroid Basis	Allethrin Equivalent Basis	Mixture on Basis of Pyrethroid Content	Pyrethroid Alone[a]		
Piperonyl butoxide plus—						
d-trans isomer	53.1±2.0	4.49	0.246±0.013	0.0846	2.91	0.92
dl-trans isomer	75.8±2.9	5.03	.172±0.0094	.0664	2.59	0.98
dl-cis isomer	92.4±3.4	5.66	.141±0.0076	.0613	2.30	1.08
barthrin	78.7±3.0	4.69	.166±0.0089	.0596	2.79	1.0
Barthrin[c]	219.0	13.05	—	.0596	—	—
Allethrin[c]	13.05	13.05	1.0	1.0	—	—

[a] Ratios calculated from DC-50's previously obtained (Gersdorff *et al.* 1959). These express expected effectiveness if mixing had resulted in no change in action.
[b] With each measure based on barthrin mixture in tables 2 and 3.
[c] Calculated as described in text.

of the four LC-50's on the allethrin equivalent basis for the barthrin ester mixtures in table 2 is 42.27. An LC-50 for barthrin alone in this study, 389.2, may be estimated from the barthrin mixture by the reverse of the above operation with the relevant figures (118.0 multiplied by 3.298). This value may be compared with 389.1, 420.3, and 398.0, obtained by a different procedure, division of 125.3 (the LC-50 for allethrin) by the three estimates obtained for the toxicity of barthrin relative to allethrin, 0.3220 and 0.2981 (Gersdorff *et al.* 1959) and 0.3148 (Gersdorff & Piquett 1959).

Evaluation of Relative Knockdown Effectiveness of the Mixtures.—To study knockdown effectiveness of the piperonyl butoxide mixtures the pertinent data in table 1 were given statistical treatment similar to that given the mortality data. The final regression equations are given below. Piperonyl butoxide alone causes only negligible knockdown in this method of application (Gersdorff *et al.* 1957), and would contribute little, if at all, to the knockdown stimulus of a mixture, except perhaps synergistically. Therefore, no equivalents were calculated and the concentrations in the equations refer to those of the pyrethroidal materials only. In the calculation of error estimates, a heterogeneity factor, 7.409, was required. The standard error of the regression coefficient was 0.188.

Piperonyl butoxide plus—	
d-trans ester	$Y=4.3423X-2.4919$
dl-trans ester	$Y=4.3423X-3.1625$
dl-cis ester	$Y=4.3423X-3.5350$
barthrin	$Y=4.3423X-3.2324$

From these equations median effective concentrations (DC-50's) were calculated; they are given in table 3 together with other pertinent calculations.

Discussion of Relative Knockdown Effectiveness.—If the toxicity and knockdown effectiveness relative to the barthrin mixture are calculated for each of the other mixtures in tables 2 and 3, the ratios for the *d-trans*, *dl-trans*, and *dl-cis* ester mixtures are, respectively, 1.62, 1.06, and 0.79 for lethal action and 1.48, 1.04, and 0.85 for paralytic action. Thus, there is no real difference relatively between the two types of measurements with these mixtures.

If the LC-50 on the pyrethroid equivalent basis is divided by the DC-50 for each mixture, the following four ratios are obtained—1.37, 1.47, 1.61, and 1.50, with a mean of 1.49. Thus, the intensity of the stimulus required to cause 50% mortality is about 1.5 that required to cause 50% knockdown. A similar ratio (1.7, which is within tolerance) was obtained for these isomers when unsynergized (Gersdorff *et al.* 1959). However, the ratio found for similar synergistic mixtures of allethrin was 2.6 and for unsynergized allethrin it was estimated to be 8.4 (Gersdorff *et al.* 1957). The similarity in the ratios for the barthrin isomers and not for allethrin implies that for the former both lethality and knockdown effectiveness are synergized equally whereas for allethrin only lethality is synergized.

With the use of two estimated DC-50's previously obtained for allethrin alone (Gersdorff *et al.* 1957) and the assumption that the DC-50's will vary in the same proportion as the LC-50's with different populations of flies, it is possible to form an opinion regarding the intensity of synergism with respect to knockdown. This assumption is certainly not unlikely when judged by results with this testing procedure. No consideration need be given the small differences in slope since the median concentrations are not far removed from the means of the test concentrations and so would be affected negligibly by a change in slope.

The estimated DC-50's for allethrin were 7.07 and 8.52 mg. per deciliter when the LC-50's were, respectively, 75.18 and 74.53. From this we deduced estimates of 11.8 and 14.3 for the DC-50 of allethrin against the flies used in this study (125.3 divided by 75.18 and multiplied by 7.07, and 125.3 divided by 74.53 and multiplied by 8.52). From the mean, 13.05, and the DC-50's of the mixtures, the knockdown effectiveness of the mixtures relative to allethrin alone may be calculated. Similarly, the knockdown effectiveness of the barthrin esters alone may be calculated with the substitution of their DC-50's and the LC-50 of allethrin as determined in their study (Gersdorff *et al.* 1959), the DC-50 for allethrin against those flies being estimated as 9.56 mg. per deciliter. As shown in table 3, the effectiveness of the mixtures divided by the effectiveness of the esters alone (the expected effectiveness if there is no synergism or antagonism) furnishes estimates of the intensity of synergism for knockdown.

These values, given in table 3, are within tolerance of the estimates of the intensity of synergism for lethality.

The DC-50's estimated for barthrin and allethrin indicate barthrin is about 6% as effective as allethrin in knockdown action. Thus, against house flies its value relative to allethrin as a knockdown agent is about one-fifth or sixth its value as a lethal agent. Since the DC-50's given for barthrin and allethrin are not obtained from direct tests but are derived by deduction with the further complication of different groups of fly populations, they were examined for consistency with some previous result obtained from actual tests. As cited above, the LC-50's of these two materials may be estimated by multiplying the DC-50's by 1.7 for barthrin and by 8.4 for allethrin, obtaining 372 and 110 mg. per deciliter, respectively. These values indicate barthrin to be 29.6% as toxic as allethrin; three direct determinations of this value were 32.2, 30.8, and 31.5% (Gersdorff *et al.* 1959).

The biometric results permit us to conclude that the reason for knockdown as measured in this testing procedure being synergized for barthrin and its isomers and not detectably for allethrin and its isomers lies in the cause for the greater disparity between the stimuli for lethality and those for knockdown with the second group of compounds. Concentration requirements appear so low with this group that initial paralysis is effected and the action completed before a threshold of synergism is reached, whereas with the barthrin group both actions occur after such a threshold.

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An Evaluation of Resistance of Corn Leaf Tissue to Feeding by European Corn Borer Larvae[1]

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ABSTRACT

Five related inbred lines of field corn were evaluated for resistance to leaf feeding by the European corn borer, *Pyrausta dubilalis* (Hubn.), using field and laboratory methods. These lines were Minnesota resistant lines A288 and A295, susceptible line A291, and their common parents, resistant line L317 and susceptible line A344. Studies reported here have shown that results obtained in laboratory studies of survival of corn borer larvae feeding on resistant and susceptible leaf tissue were generally similar to results obtained in field studies.

This work has shown that the resistance present in the lines studied was due primarily to antibiosis. It has also shown that there are differences in survival of newly hatched corn borer larvae reflecting differences in planting date, stage of growth of the plants, and maturity of corn leaf tissue, as well as differences in overall plant resistance. Although care must be taken whenever data obtained in the laboratory are compared with field data and data obtained in other types of laboratory studies, the available evidence indicates that the laboratory technique employed will prove useful as another tool in furthering our understanding of resistance. This technique has the advantage that small samples (1½ inches by 3 inches) of leaf tissue from areas of the plant where resistance is most effective may be evaluated for presence of resistant factors. The use of related inbred lines has demonstrated some of the genetic implications of resistance studies.

The European corn borer, *Pyrausta nubilalis* (Hubn.), is responsible for considerable loss in yield of corn each year in the United States. One of the methods of reducing losses is the use of varieties of corn resistant to corn borer attack. This paper describes a laboratory technique that has been developed for the study of resistance to feeding of the borer on corn leaf tissue. It also gives an evaluation of resistance of five related inbred lines of corn made with a series of field studies and an evaluation of the same five inbred lines made in parallel studies with the laboratory technique.

Most of the earlier studies on resistance of corn to borer attack were made in the field with either natural or artificially induced borer infestations. Only recently

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have attempts been made to experiment in the laboratory under controlled conditions for better understanding of the nature of resistance.

Patch (1943) compared differences in survival, weight, and location of corn borer larvae feeding on resistant and susceptible corn under field conditions. He found that during the first 4 days after hatching, survival of larvae feeding on resistant corn was much less than survival of larvae feeding on susceptible corn. After this initial period, there was little difference in survival of larvae. First-instar larvae feeding on susceptible corn plants ate long, narrow portions of leaf tissue in contrast to the small pin-point type of larval feeding found on resistant plants. Larvae developed faster and weighed more when fed on plants of susceptible corn than when fed on plants of resistant corn.

Bell (1956) suggested that differences in resistance of some inbred lines may be caused by differences in cellular structure of the upper epidermis of the corn leaf. Resistant lines studied had small groups of bulliform cells, while susceptible lines studied had larger groups of bulliform cells. He found that corn borer larvae initiated feeding on these groups of bulliform cells. Differences in staining reactions of the bulliform cells of resistant and susceptible lines indicated possible differences in chemical constituents of the cells. His original thesis Bell (1954) stated the differentiation of leaf tissues in the whorl began at a lower level in plants of susceptible lines than in plants of resistant lines.

Numerous studies have shown that in many lines of corn the expression of resistance varies according to the stage of growth of the corn plant. Patch (1937) and Schlosberg & Baker (1939) infested corn in different stages of growth with corn borer egg masses in order to study differences in larval survival owing to differences in plant maturity at the time of infestation. They found that survival of larvae increased with maturity of the plant. Similarly, Luckmann & Decker (1952) found that survival of larvae increased with an increase in the tassel ratio, which was used as an index of plant maturity.

The general kind of resistance in the studies reported here is that concerned with initiation and continuation of larval feeding. The problem of resistance of corn to borer attack is complicated, however, by the existence of several types of resistance to larval feeding. Differences in resistance may be present in the leaf blade, midrib, ligule, collar, sheath, tassel, stalk, husk, ear, or ear shank. Larvae feed on different parts of the corn plant according to the size of the larvae and the stage of growth of the plant. Resistance factors present in restricted parts of the plants may be effective only against larvae feeding on those parts of the plants. Resistance may not be effective owing to a lack of coordination of the larval feeding cycle with the location of factors for resistance. Most work on resistance has emphasized resistance of leaf tissue to feeding by first-instar larvae of the first brood. In later instars, larvae of the first brood have been shown by Patch (1943), to feed in the tassel, leaf midrib, ligule, collar, sheath, and finally in the stalk. Larvae of the second brood feed chiefly in the sheath, stalk, ear, and ear shank.

Recently, several laboratory studies have been conducted with the European corn borer. Bottger (1940, 1942) studied the nutritive requirements of corn borer larvae. Beck *et al.* (1949) developed an artificial medium to be used as food for corn borer larvae. Recently an aseptic rearing method (Beck & Stauffer 1950) has been used in laboratory investigations of nutritional factors affecting survival of the corn borer (Beck 1956a, 1956b).

Beck & Lilly (1949) allowed newly hatched corn borer larvae to feed on excised corn leaf tissue for 6 days. The leaf tissue was replaced after 3 days with fresh leaf material. Mortality and weight of larvae were determined after 6 days. These authors found that mortality of larvae was lower and larvae weighed more when fed on leaf tissue from taller plants than on shorter plants. They also found that mortality of larvae was higher and larvae weighed less when fed on leaf tissue from resistant plants than on tissue from susceptible plants. Chin (1951) fed excised leaf tissue from resistant and susceptible corn plants to corn borer larvae. He replaced the tissue with fresh material at intervals of 2 days. The larvae were reared to maturity in this manner. He found that larval mortality was less, larvae consumed more leaf tissue, and larvae gained more weight and faster, when fed leaf tissue from susceptible corn than tissue from resistant corn. He also found that larvae initiated feeding more readily on leaf tissue from susceptible than from resistant inbred lines.

This paper concerns the development of a laboratory method for evaluating resistance of corn to leaf feeding by corn borer larvae, as well as field studies carried on at the same time as the laboratory studies, to evaluate resistance under natural conditions. Studies were made of differences in resistance of five inbred lines of corn, of differences in resistance of plants in the midwhorl and late-whorl stages of growth, and of differences in survival of larvae feeding on immature and mature leaf tissue.

PROCEDURES.—On the basis of preliminary experiments, several related inbred lines were selected for inclusion in this research. These lines were Minnesota lines A288, A291, and A295 and their parent lines, Minnesota A344 and Iowa L317. Lines A344 and A291 have been rated by Minnesota agronomists as susceptible, and lines L317, A288, and A295 as resistant to leaf feeding by larvae of the European corn borer. Since these lines are related, it would seem probable that factors for resistance are the same in all the lines that are resistant to borer attack.

The corn used in these studies was planted at University Farm, St. Paul, on May 21, June 2, and June 12, 1954. The inbred lines were grown in a randomized block with each experiment replicated either three or five times within planting dates; the May 21 and June 12 plantings were replicated three times and the June 2 planting was replicated five times. At regular intervals throughout the season growth records were obtained for plants of each line and each planting date. Measurements recorded included the normal height (distance from the base of the stalk to the uppermost point of the plant in its normal growing position) and extended height (distance from the base of the stalk to the tip of the leaves when extended) of each plant. The number of mature leaves, the number of visible leaves and the stage of growth (Batchelder 1949) were also recorded for each plant. The third and fifth leaves of the plants were marked in order that

the number of leaves present could be determined more readily.

Field studies were carried out to determine the type and amount of corn borer damage, as well as survival of larvae. Plants of each line were artificially infested with corn borer egg masses on several dates to obtain information regarding survival of larvae on plants infested in the "midwhorl" and "late whorl" stages of growth. The normal height and extended height of each plant were recorded at the time of infestation. Between 6 and 7 weeks after infestation, the normal height and extended height of each plant were again measured to determine if plant growth had been retarded due to the borer infestation. Infested plants were rated for first-brood injury by estimating leaf injury and counting damage points. These ratings were based on a system developed by F. F. Dicke. A leaf injury rating of 1 indicated no corn borer injury and a leaf injury rating of 5 indicated severe leaf feeding by corn borer larvae. All damage points over one-half inch in length were counted. Plants were then dissected and the living, dead, and parasitized larvae, as well as empty tunnels, were counted.

During the summer of 1953, a technique was developed for laboratory studies of survival of corn borer larvae feeding on corn leaf tissue. This technique was used in 1954 to evaluate differences in survival of corn borer larvae feeding on resistant and susceptible lines of corn. Plants to be evaluated by this technique were selected for uniformity of size and growth characteristics from the plants available in each plot. Plants were cut at the soil surface and taken to the laboratory where the normal and extended height were recorded. The leaves were removed and subdivided into sections, each 3 inches long, beginning with a section immediately below the level of moisture in the cup of the whorl of leaves. (Fig. 1 and fig. 2.) Twelve discs of leaf tissue were cut from each section of the leaf using a $\frac{1}{2}$-inch punch mounted in a drill press. As they were cut, the discs fell through the die onto a strip of heavy paper. Operation of the punch is illustrated in figure 3. A leaf with a set of discs cut from one side of a section of leaf tissue is shown in figure 4. Discs were also cut from areas immediately above and immediately below that shown in the figure, as well as from similar areas on the other side of the leaf midrib. The 12 discs from each section of leaf tissue were divided into 2 samples of 6 discs each. The six discs were placed on a straight pin and put in a vial (fig. 5). A one-hole rubber stopper, plugged with cotton, was used to close the mouth of the vial. The selection and randomization of discs of leaf tissue was based on a 12×12 Latin square design.

Corn borer egg masses were selected at random for uniformity of size from stocks of egg masses being produced in the laboratory. Thus, in any one experiment differences in survival represent differences in the leaf tissue being studied. No effort has been made to study possible differences in borer populations by this method. The original stock of overwintering larvae was obtained from field corn in southwestern Minnesota. The eggs were stored at constant temperature and humidity until just before hatching. At this time the viable eggs in each mass were counted and the egg mass was placed in a vial containing discs of leaf tissue. Use of egg masses instead

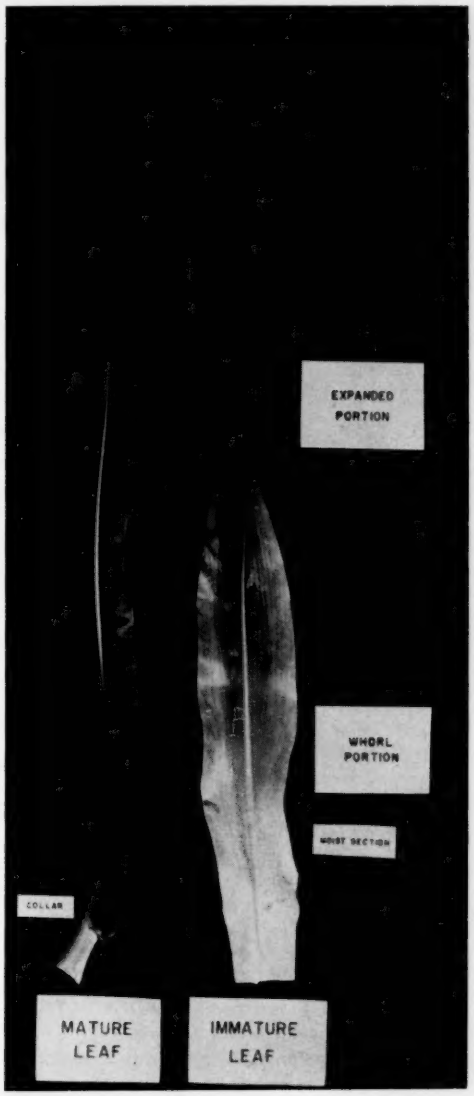

FIG. 1.—Mature and immature leaves of corn showing differences in appearance of the leaves as well as portions of leaf tissue involved in these studies.

of newly hatched larvae was found to save much time by eliminating the need for handling the larvae. With this system, however, the initial number of larvae in each vial could not be kept constant. The initial number of larvae per vial, in the 1954 series of tests, was 26.9 ± 3.7. Vials containing discs of corn leaf tissue and corn borer egg masses were kept at 27° C. until the eggs had hatched. They were then transferred to a chamber at 20° C. and 80% relative humidity for 96 hours. At this time the hatched eggs and surviving larvae were counted.

Data obtained in these experiments were in the form of a binomial, that is,

$$p+q=1$$

where p is the proportion of surviving larvae and q is the proportion of dead larvae. For this type of data, the variance is dependent upon p and q, as well as upon n, the

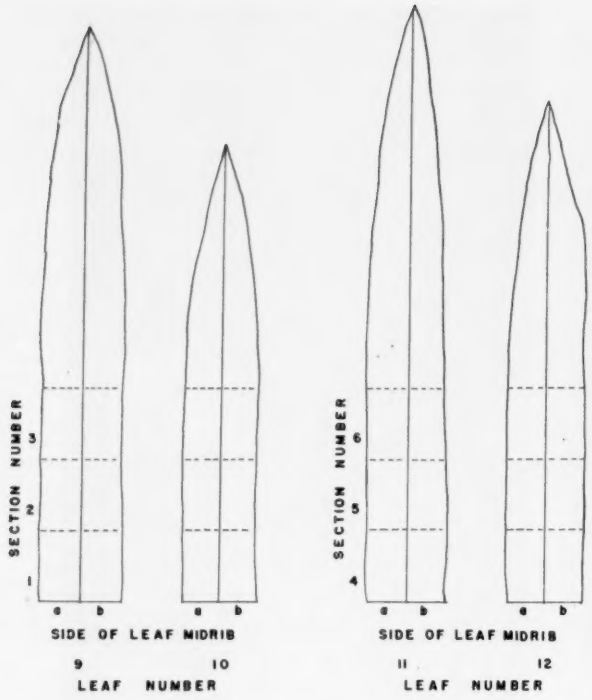

FIG. 2.—Corn leaves illustrating the method of sampling leaf tissue from plants in the midwhorl stage (leaves 9 and 10) and from plants in the late-whorl stage of growth (leaves 11 and 12). Two samples were taken from each side of the midrib (a and b) of each section of each leaf listed for a total of 24 samples of tissue from each leaf.

FIG. 3.—Operation of punch for cutting discs of corn leaf tissue.

initial number of larvae present. The variance would be:

$$\frac{pq}{n}$$

(Mather 1947). Since the variance is dependent upon the observation, a transformation of the original data is required. The appropriate transformation in this instance is the inverse sine transformation:

$$p = \sin^2 \phi$$

as proposed by Bliss (1937, 1938). If no heterogeneity is present between plots, the variance of the transformed data is theoretically $820.7/n$, when the angle is measured in degrees. This fact is dependent only upon n, which is usually a constant.

As mentioned earlier, n was not a constant for these experiments. Therefore, an investigation was devised to determine if differences in n had any effect upon the results of the experiments. The procedures for this group of tests were similar to those employed in the laboratory method described in the preceding pages with the exception that an egg mass containing a large number of eggs (40.9 ± 4.1) was placed in the vial containing one sample of six leaf discs, and an egg mass containing a relatively small number of eggs (24.0 ± 3.4) was placed in the vial containing the second sample of six leaf discs cut from each area of the plant. The survival of corn borer larvae in this series of experiments was determined and the data were transformed to angles and subjected to an analysis of variance to determine if differences in n were significant. If analysis of these unweighted data showed the presence of possible differences owing to n, use of weighted data would be necessary to correct for these differences. However, if differences owing to n were not significant for unweighted data, weighting would not be necessary. As will be noted later, weighting of the data was unnecessary.

GROWTH AND RESISTANCE OF CORN UNDER FIELD CONDITIONS.—Studies were made of the growth and resistance of an early (May 21) and a late (June 12) planting of five inbred lines of corn during the summer of 1954. Five plants from each plot were measured at approximately 4-day intervals. The normal height, extended height, and stage of growth were recorded for each plant. Plants of the early planting were in the midwhorl and late whorl stages of growth during the period from June 29 to July 14. Plants of the late planting were in these same stages of growth during the period from July 8 to July 25. Patch (1943) has shown that differences in resistance of corn plants to leaf feeding by first instar corn borer larvae of the first brood are found in plants that are in the midwhorl and late whorl stages of growth.

Differences in height of inbred lines are summarized in table 1. These data show that under Minnesota conditions the parental line A344 grows faster than line L317 but, since the leaves of L317 are longer, the extended height

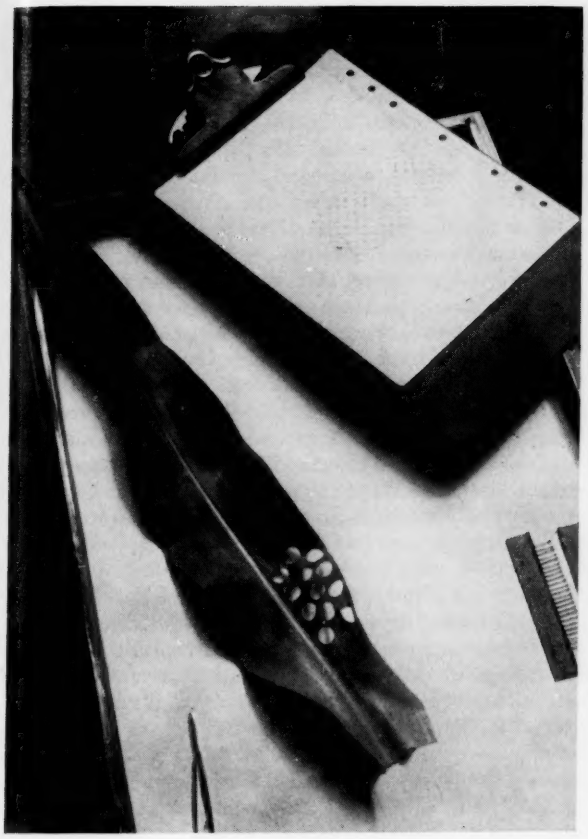

FIG. 4.—Corn leaf with discs of leaf tissue removed from the area of the moisture level.

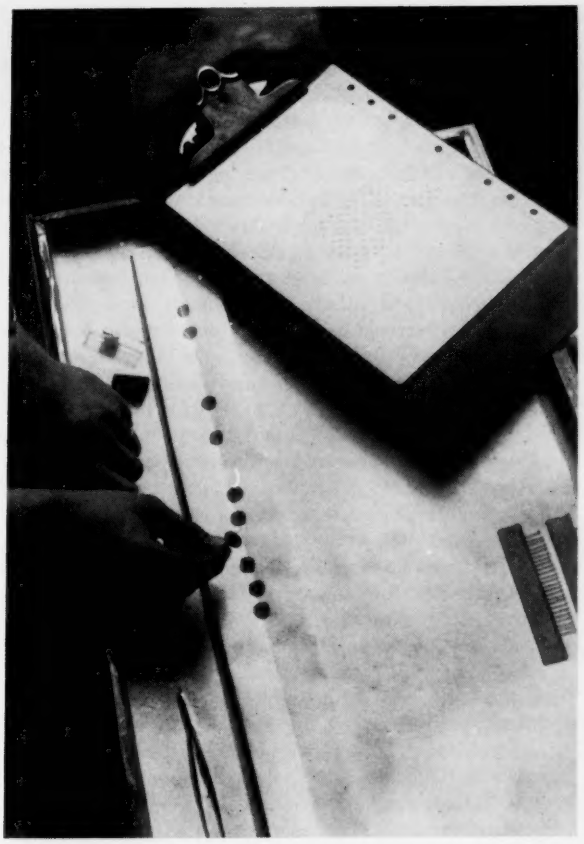

FIG. 5.—Selecting and pinning discs of leaf tissue to be fed to newly hatched corn borer larvae.

of L317 approaches and exceeds that of A344 during this period of growth. Mature plants of line L317 were much taller than those of line A344 because of the longer growing period of the former. Of the progeny lines, the susceptible, A291, was taller than the resistant, A288 and A295, during the whorl stages of growth. However, these differences in height were not consistently significant and, on several dates, there were significant differences between the heights of the sister resistant lines, A288 and A295, but not between the taller of these lines and its sister susceptible line, A291. Thus, differences in height of corn on a given date were not consistently related to differences in resistance rating.

Table 1.—Height of uninfested corn plants measured during the midwhorl and late-whorl stages of growth.

TIME OF PLANTING	DATE MEASURED	RESISTANT LINES			SUSCEPTIBLE LINES		L.S.D. (5%)
		L317	A295	A288	A291	A344	
		Average normal height (inches)					
Early	June 29	12.8	11.8	14.6	13.6	13.8	ns
	July 6	19.0	17.0	20.4	21.6	20.2	ns
	July 14	30.8	26.8	32.4	34.2	35.4	4.2
Late	July 8	12.8	14.6	13.0	14.8	14.2	ns
	July 17	20.6	24.4	20.8	24.8	24.2	2.7
	July 25	29.6	34.2	32.4	38.2	40.6	4.7
		Average extended height (inches)					
Early	June 29	22.9	18.0	21.1	23.0	20.6	3.1
	July 6	32.8	25.8	29.4	31.8	29.2	3.5
	July 14	46.0	35.2	42.2	45.6	42.0	3.8
Late	July 8	20.4	20.4	18.2	21.8	21.4	2.2
	July 17	34.2	32.4	30.8	36.8	33.4	2.9
	July 25	44.8	42.0	41.6	49.4	46.0	3.8

Availability of the tassel as a site for larval feeding has been shown to contribute to larval survival (Patch 1943). Differences in the time of tasselling of the inbred lines were studied to determine if these differences could be related to differences in resistance of these lines to corn borer attack. The stage of growth of plants of each line was determined at intervals during the growing season. From these data the average date of tassel appearance was calculated (table 2). Plants of the susceptible parent line, A344, tasselled earlier than plants of the progeny lines, A288, A291, and A295. Plants of the resistant parent line, L317, tasselled later than plants of the other lines. Differences in tasselling of the parent lines are expected, owing to differences in the time required for these lines to reach maturity. In the early planting, plants of the susceptible progeny lines, A291, tasselled earlier than

Table 2.—Date of tassel appearance of early- (May 21, 1954) and late- (June 12, 1954) planted corn of five inbred lines.

TIME OF PLANTING	DATE OF TASSEL APPEARANCE (1954)				
	Resistant Lines			Susceptible Lines	
	L317	A295	A288	A291	A344
Early	August 8	July 31	July 27	July 24	July 20
Late	August 18	August 9	August 8	August 8	July 29

plants of the resistant progeny lines. However, in the late planting there were no differences in date of tasselling.

Differences in damage caused by European corn borer larvae feeding on resistant and susceptible inbred lines of corn were studied by an artificial corn borer infestation. Corn planted on May 21, June 2, and June 12 was hand infested with corn borer egg masses when the plants were either in the midwhorl or the late whorl stages of growth. Damage caused by corn borer larvae feeding on the artificially infested plants was evaluated by determining reduction in height of the mature plants and by rating infested plants for "leaf injury," "damage points," and "corn borer index" about 7 weeks after the plants had been infested. Two systems in common usage for estimating resistance to corn borer injury are: (a) rating plants for leaf injury and, (b) for damage points. The "corn borer index," developed as a result of the studies reported in this paper, and proposed here for the first time, is obtained by summing the total number of tunnels per plant, the number of full-grown larvae that have died, and the larvae found on the outside of the stalk. The corn borer index is roughly equivalent to the total number of tunnels per plant, used by some workers, but is considered by the present authors to be a more accurate measure of injury than is the number of tunnels alone. Data for all plants infested in each stage of growth (midwhorl and late whorl) were averaged to obtain the summary presented in table 3.

The maximum normal height of infested corn was compared with the maximum normal height of uninfested corn to determine the percentage reduction in height due to borer infestation. The greatest reduction in height occurred in plants of the susceptible lines infested in the midwhorl stage of growth (table 3).

The leaf injury rating of plants of lines L317, A295, and A288 infested in the midwhorl stage of growth indicated that these lines were resistant to leaf feeding, while the leaf injury ratings of similar plants of lines A291 and A344 indicated that they were susceptible to leaf feeding by corn borer larvae. The leaf injury rating of plants of all lines infested in the late whorl stage of growth indicated little or no difference in resistance of these lines to leaf feeding by corn borer larvae when the plants are infested in this stage of growth. This may be explained partially by the fact that leaf tissue of plants in the late whorl stage of growth was used by larvae as food for only a few days before the tassels became available as food for the larvae. Therefore, the larvae did not cause conspicuous leaf injury.

Table 3.—Reduction in height and damage to artificially-infested plants of five inbred lines of corn caused by corn borer larvae.

Stage of Growth at Infestation	Inbred Line				
	Resistant Lines			Susceptible Lines	
	L317	A295	A288	A291	A344
	Percentage reduction in normal height				
Midwhorl	0	2.3	1.4	15.1	17.4
Late-whorl	0	0.0	2.5	0.8	7.6
	Leaf injury rating[a]				
Midwhorl	1.4	2.1	2.0	4.6	3.8
Late-whorl	1.5	1.4	1.6	2.0	1.3
	Number of damage points				
Midwhorl	3.1	2.5	1.2	7.2	5.0
Late-whorl	3.3	2.3	3.4	3.4	6.3
	Corn borer index				
Midwhorl	2.1	2.1	1.8	3.3	4.4
Late-whorl	3.7	3.6	7.6	6.9	11.5

[a] Rating of 1 indicates no injury, 5 most severe injury.

The evidence here also indicates the necessity for infesting plants that are to be rated for leaf injury not later than the midwhorl stage if a satisfactory evaluation of resistance to leaf feeding injury is to be obtained.

More damage points were found on plants of the susceptible lines, A291 and A344, infested in the midwhorl stage of growth than on plants of the other lines infested in the same stage of growth. Plants of line A344, infested in the late-whorl stage of growth had a higher number of damage points than plants of other lines infested in the same stage of growth.

The corn borer index was larger for plants of all lines infested in the late whorl stage of growth than for plants infested in the midwhorl stage of growth. The corn borer index was larger for plants of the susceptible lines, A291 and A344, infested in the midwhorl stage of growth than for plants of resistant lines, L317, A295, and A288, infested in the same stage of growth. The index for plants of the susceptible lines, A291 and A344, and one resistant line, A288, infested in the late whorl stage of growth was larger than the index for similar plants of the other resistant lines, L317 and A295.

Studies of differences in resistance of related inbred lines of corn to feeding by corn borer larvae indicated that somewhat different results were obtained by each of the methods used to evaluate corn for resistance. When infestation occurred in the midwhorl stage of growth, damage to plants of the susceptible lines, A291 and A344, was more severe than damage to plants of the resistant lines, L317, A295, and A288. However, variations were evident in the degree of differences obtained by each method. The difference in borer population on the resistant lines compared with the susceptible lines resulted in a much greater difference in damage when measured by growth of the corn than when measured by either leaf injury or damage points. (Further information on this kind of injury is reported by Chiang & Holdaway (1959).) Damage, as measured by percentage reduction in height, of the susceptible lines was 14 times that of the resistant lines, but, when measured by the other methods, damage to susceptible lines amounted to two or three times that of the resistant lines. Therefore, in addition to a lower survival of borers on the resistant lines, these lines are able to tolerate the population present with much less injury to the plants than are susceptible lines.

Rating plants infested as late as the late-whorl stage of growth indicated that the various methods used to determine differences in borer infestation and damage did not give consistent results. Use of leaf-injury rating indicated no differences in borer damage on resistant and susceptible lines. Use of either the number of damage points or the reduction in height indicated that one susceptible line, A344, was damaged more severely than

the other susceptible line, A291, and the three resistant lines. The borer population, as measured by the borer index, was largest on susceptible line A344, and lowest on resistant lines L317 and A295. The borer index for susceptible line A291, was about the same as that of resistant line A288. The inconsistency of the results obtained by these various methods may be related to the fact that larvae feeding on plants in the late whorl stage of growth prefer to feed on the tassel instead of leaf tissue as soon as the tassel is available as a source of food.

PROCEDURES FOR EVALUATION OF RESISTANCE IN THE LABORATORY.—Several experiments were devised to determine the best procedures for further studies of resistance under laboratory conditions. Results of a few of these are useful for interpreting the results of studies of resistance in the laboratory and will be reported here.

A study was undertaken to determine the maximum time that newly hatched corn borer larvae could survive without food under conditions similar to those which were used later in studies of survival of corn borer larvae feeding on corn leaf tissue. Except for omission of the discs of corn leaf tissue, experimental conditions for these studies were the same as conditions described previously. The humidity within these vials probably was lower than the humidity within vials containing discs of corn leaf tissue owing to the absence of moisture from the leaf tissue. Sixty vials containing newly hatched corn borer larvae were used in tests to determine survival of starved larvae. Ten of the 60 vials were selected at random every 12 hours and the percentage of surviving larvae was determined. These data (table 4) show that, although a few larvae were able to survive 60 hours, no newly hatched larvae were able to survive 72 hours without food. The mean survival time for larvae was 46 hours. These results were similar to those found by Tauber & Bruce (1946).

Studies of survival of larvae feeding on corn leaf tissue for either 72 or 96 hours were conducted to determine differences in survival caused by differences in length of the test period. Occasionally the food supply, consisting of six discs of leaf tissue placed in each vial, was practically exhausted after a period of 96 hours. Therefore 96 hours was the maximum length of test period considered. Comparisons of survival of corn borer larvae feeding on corn leaf tissue for periods of 72 and 96 hours were made by dividing the 12 discs cut from each area of leaf tissue into 2 samples of 6 discs each for testing as described previously. Survival of larvae in one sample of each pair was determined 72 hours after hatching and in the other sample 96 hours after hatching. These experiments showed that more than 90% of the larvae survived a test period of 72 hours. Between 68% and 80% of the larvae survived a test period of 96 hours. A test period of 96 hours was found necessary to show differences in survival of larvae feeding on different types of corn leaf tissue. This was to be expected since larvae could survive almost 72 hours without food.

Differences in survival of corn borer larvae caused by crowding and competition were also studied. Twelve discs of leaf tissue cut from each area were divided into 2 samples of 6 discs each. An egg mass containing only a few eggs per mass (24.0 ± 3.4) was placed in a vial with one sample of leaf discs from each pair, and an egg mass containing a larger number of eggs per mass (40.9 ± 4.1) was placed in another vial with the second sample of leaf discs. Survival of larvae was determined 96 hours after hatching. Although such survival (64.3%) appeared to be somewhat lower where crowding was more severe than in vials where crowding was less severe (73.4%), these differences were not significant. Since differences in initial number of larvae in these tests were much greater than in subsequent tests, it was assumed that differences in initial population in subsequent tests did not influence the results obtained. In later tests, the initial number of larvae present was 26.9 ± 3.7.

SURVIVAL OF LARVAE FEEDING ON EXCISED LEAF TISSUE.—Survival of newly hatched first-instar larvae feeding on leaf tissue taken from plants of five related inbred lines was studied to decide if differences in resistance as determined under laboratory conditions were similar to differences in resistance to leaf feeding by corn borer larvae as determined by field tests. Plants from an early (May 21) and a late (June 12) planting of resistant lines L317, A295, and A288, and susceptible lines A291 and A344, were included in these studies. One group of tests was conducted using leaf tissue cut from plants in the midwhorl stage; a second series of tests was conducted using leaf tissue cut from plants in the late whorl stage. All five lines were included in tests of plants in the midwhorl stage but only lines A288, A291, and A295 were included in tests of plants in the late-whorl stage.

Three plants of each inbred line were included in each test. Comparable areas of two leaves from the whorl of each plant were selected and divided into sections. The center sections included the moisture level. Other sections were located immediately above and immediately below the moisture level. Twenty-four samples of leaf tissue, 2 from each of the 12 sections, were tested for each plant. Thus, 72 samples of leaf tissue were tested from each inbred line in a series.

Survival of corn borer larvae feeding on each sample of leaf tissue was calculated and transformed for analysis, using the inverse sine transformation. The average larval survival was calculated for each inbred line (72 samples). These data are presented in tables 5 and 6.

Table 5 presents the average survival of larvae fed tissue from adjacent sections of leaves from plants of resistant and susceptible inbred lines of corn planted early and late and in the midwhorl and late-whorl stage. These data indicate that the technique is one which yields results of considerable precision in measuring the susceptibility of different tissues to injury by the borer.

Table 4.—Survival of starved corn borer larvae at 20° C. and 80% relative humidity.

TIME (HOURS)	INITIAL NUMBER OF LARVAE[a]	NUMBER OF SURVIVING LARVAE	PERCENTAGE SURVIVAL
12	263	260	98.9
24	240	233	97.1
36	258	220	85.3
48	258	116	45.0
60	255	15	5.9
72	249	0	0.0

[a] From 10 egg masses, placed in individual vials.

Table 5.—Survival of corn borer larvae fed tissue from adjacent sections of leaves from plants of resistant and susceptible inbred lines of corn.

Time of Planting	Inbred Line	Rating	Larval Survival (%) Below Moisture Level	Moisture Level	Above Moisture Level	Significance of Difference Between Sections[a]
Plants tested in the midwhorl stage of growth						
Early	L317	Res.	94.4	93.7	91.3	ns
	A295	Res.	94.3	86.3	86.0	ns
	A288	Res.	94.5	93.9	83.5	*
Average resistant			94.4	91.3	86.9	
	A291	Susc.	98.3	94.9	95.2	ns
	A344	Susc.	93.7	94.8	95.7	ns
Average susceptible			96.0	94.8	95.4	
Mean for early planting			95.2	93.0	90.9	
Late	L317	Res.	81.0	61.6	53.5	*
	A295	Res.	92.3	84.6	65.8	***
	A288	Res.	93.0	84.0	34.4	***
Average resistant			88.8	76.7	51.2	
	A291	Susc.	95.7	89.4	76.7	*
	A344	Susc.	93.8	76.5	64.5	***
Average susceptible			94.8	82.9	70.6	
Mean for late planting			91.7	79.9	59.2	
Plants tested in late-whorl stage of growth						
Early	A295	Res.	85.1	65.5	76.5	*
	A288	Res.	92.3	67.8	42.0	***
Average resistant			88.7	66.6	59.2	
	A291	Susc.	93.2	67.6	84.9	**
Mean for early planting			90.4	66.9	67.8	
Late	A295	Res.	88.5	38.5	35.9	***
	A288	Res.	93.3	56.8	23.7	***
Average resistant			90.9	47.6	29.8	
	A291	Susc.	96.2	71.5	68.9	***
Mean for late planting			93.0	55.7	42.8	

[a] Differences in survival of larvae fed tissue from different locations, within lines and plantings, significant at the 5% level*, 1% level**, and 0.1%***.

Differences in survival of borer larvae fed tissues of the respective lines with the level of significance of the data are summarized in table 6, while differences in survival of borer larvae fed tissue from different locations within lines and plantings are given in table 5.

The data show that survival of larvae fed on leaf tissue from susceptible lines of corn was usually higher than survival of larvae fed on resistant lines. Survival of larvae fed on corn in the midwhorl stages of growth was higher than survival of larvae fed on corn in the late whorl stage of growth. Also, survival of larvae fed on early-planted corn was higher than survival of larvae fed on late-planted corn. Analyses of the original data show that differences in survival of larvae were not related to either the date or time of day that tests were initiated.

Differences in survival of newly hatched corn borer larvae feeding on tissue cut from different parts of corn plants had been suggested by preliminary studies. Analyses were made of the data from individual corn plants to determine if significant differences occurred in survival of larvae feeding on different parts of these plants. These analyses showed that differences in survival of larvae feeding on adjacent leaves or on opposite sides of leaves were not significant. However, differences in survival of larvae feeding on tissue from the moisture level and from above and below this area of plant tissue were significant for 83% of the plants tested in the late-whorl stage and for 47% of those tested in the midwhorl stage. (table 5). These differences were found in all five lines of corn tested. In general, survival was higher when larvae were fed tissue from below the moisture level than tissue from the moisture level or above. These findings are similar to those reported by Bell (1954) in morphological studies of corn plants as related to borer survival. Beck (1956a)

Table 6.—Survival of born borer larvae fed leaf tissue from plants of resistant and susceptible inbred lines of corn. Summary table with significance of the data.

Stage of Growth	Planting	Larval Survival (%) Resistant Lines L317	A295	A288	Susceptible Lines A291	A344	Significance of Difference[a]
Midwhorl	Early	93.2	89.3	91.2	96.4	94.7	ns
	Late	66.0	82.1	74.3	88.3	79.8	*
Late-whorl	Early		76.1	69.6	83.1		*
	Late		56.1	60.4	81.1		*

[a] Differences in larval survival between lines, within plantings, significant at the 5% level indicated by *.

reported that the total sugar content of the basal portion of whorl leaves was 19.2 mg. per gram fresh weight compared with only 11.4 mg. per gram for the middle portion of whorl leaves. Under field conditions, larvae feed on the basal portion of the whorl leaves in preference to the middle portion. However, newly hatched larvae are generally unable to penetrate the moisture level to the tightly rolled section of tissue below the moisture level.

DISCUSSION.—Resistance of corn plants to attack by European corn borer larvae has been the subject of research over the past 30 years. Most of these studies employed natural or artificial corn borer infestations under field conditions. Until recently little work had been done in laboratory studies of resistance of corn to larval feeding under constant controlled conditions. Bottger (1940, 1942) and Beck, *et al.* (1949) developed artificial media for rearing corn borer larvae in the laboratory. Beck (1956a, 1956b) and George (1958) have employed artificial media in physiological studies of resistance of corn to corn borer feeding.

Beck & Lilly (1949) and Chin (1951) studied differences in mortality of corn borer larvae feeding on excised corn leaves under laboratory conditions. Their findings showed that mortality of larvae feeding on leaves of resistant plants was higher than mortality of larvae feeding on leaves of susceptible plants. Larvae reared on leaves of susceptible plants were also found to weigh more than larvae reared on leaves of resistant plants. In each of these studies, fresh leaf material was supplied to the larvae at intervals of 2 or 3 days. Evidence was found that the concentration of factors responsible for resistance varies not only in different plant tissues but in similar tissues of different plants. Therefore, fresh leaf tissue supplied to the larvae may not contain a similar amount of factors for resistance as was contained in the original tissue fed to the corn borer larvae.

The laboratory method used in the present study for evaluating resistance of leaf tissue to survival of corn borer larvae has the advantages that the tissue used is from small areas of corn leaves, and that the initial amount of tissue supplied to the larvae is sufficient for the entire test period. It has the disadvantage that the excised leaf tissue used is subject to physiological changes during the test period. The possible influence of these physiological changes in the leaf tissue cannot be disregarded. However, results obtained using this method were quite striking, and, in general, they were found to agree with results obtained in field and laboratory studies by other workers. Also, the fact that all the larvae feeding

on some of the samples of leaf tissue taken from susceptible lines were able to survive and become second-instar larvae within the 96-hour test period indicated that the rearing part of this technique was satisfactory. About 5,000 samples of excised leaf tissue have been fed to 125,000 corn borer larvae over a 2-year period using this technique.

Resistance of the three resistant lines of corn included in these experiments, L317, A295, and A288, could be due to either "pseudoresistance" or true resistance, as defined by Painter (1951). Field studies were conducted to determine some of the characteristics of the resistance present in these lines of corn. Plants of these three resistant lines and of the two susceptible lines, A291 and A344, were artificially infested with corn borer egg masses to insure a uniform initial borer population. This technique eliminates the possibility of "pseudoresistance" caused by "plant escape" or "host evasion." Resistance owing to "preference" is also eliminated in this manner. Field techniques employed were designed to eliminate any possible extraneous factors that could cause "induced resistance." The remaining possible causes of resistance were "antibiosis" and "tolerance." Although the field data were not conclusive, they indicated that resistance was due primarily to "antibiosis." Further investigations conducted to determine if any relationship could be found between differences in plant height or date of tassel eclosion and resistance showed no consistent relationship between either of these factors and differences in resistance of corn under field conditions in these particular studies.

Laboratory experiments indicated that differences in resistance of corn to corn borer larvae could be measured by feeding excised corn leaf tissue to the larvae. Survival of larvae feeding on tissue from susceptible lines A344 and A291 was usually higher than survival of larvae feeding on tissue from resistant lines L317, A295 and A288. These results agree with observations made on artificially infested plants in the field.

Survival of larvae feeding on leaf tissue from plants in the midwhorl stage of growth was found to be somewhat higher than survival of larvae feeding on tissue from plants in the late whorl stage. This is the reverse of the situation generally found under field conditions, where larval survival is higher on plants infested in a more mature stage of growth. This may be explained by the fact that, under field conditions, larvae on plants in the late whorl stage of growth prefer to feed on the young tassel as soon as it is available. In laboratory tests, however, the larvae were restricted to feeding on small areas of excised leaf tissue.

Detailed study of laboratory results showed that survival of corn borer larvae feeding on the less differentiated leaf tissue from below the moisture level was higher than survival of larvae feeding on the more mature leaf tissue from above the moisture level. Bell (1956), in making histological studies of corn, reported that differences occurred in leaf tissue of resistant and susceptible lines of corn. He found that larvae initiated feeding on the bulliform groups of cells and that bulliform cells of resistant lines were smaller than those of susceptible lines. In his original thesis (1954) he reported that differentiation of leaf tissue began at a lower level in plants of susceptible lines than in those of resistant lines. Under field conditions larvae prefer to feed on the less differentiated tissue of the moisture level than on the differentiated tissue from above the moisture level. However, larvae usually are unable to penetrate to the undifferentiated leaf tissue below the moisture level. This preference for feeding site has been shown (Beck 1956a) to be caused by a combination of borer response to light, contact, sugars, and possibly moisture.

No conclusive results have been obtained as to whether the lower survival of corn borer larvae feeding on certain types of leaf tissue was due to morphological or physiological factors or to a combination of these and possibly other factors. There are indications that death of larvae was due to repellency followed by starvation since very little tissue was eaten in tests showing low survival of larvae. The possibility of toxic or partially toxic effects has not been entirely eliminated.

While the field evaluation of resistance to the borer has yielded valuable practical developments, it is apparent that the laboratory technique described in this paper has considerable promise in elucidating many aspects of resistance that cannot be determined by the field technique alone.

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A Quantitative Measure of Aggregation in Insects[1]

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ABSTRACT

Aggregation is both a statistical and fundamental biological characteristic of insects and other animals. The aggregative behavior of nonsocial as well as social insects has a direct bearing on reproduction and survival and thus affects population dynamics and evolutionary trends. Owing to the distinct lifestages of most insects, aggregation must be assessed throughout the entire lifecycle.

The occurrence of insects in natural units of their habitats is best expressed as a frequency series. The negative binomial distribution has proved applicable to a wide range of insect counts, and its parameter k is a valid, readily computed measure of aggregation. Examples from hypothetical and natural populations demonstrate its utility.

Use of the smallest feasible natural unit for sampling, multiple (nested) sampling, and stratification of the data by population density and other meaningful criteria will increase the accuracy and reliability of estimates of k.

Most insects rarely, if ever, disperse themselves in a purely random manner in their natural environments. This is not surprising, as few environments are truly homogeneous and the behavioral responses that govern the dispersion of insects are usually specific rather than random. In general, the departure from randomness is in the direction of aggregation, or grouping.

Aggregation in insects, as with other animals, appears to be a fundamental trait with complex origins. Needless to say, it has considerable ecological significance. Aggregation ranges from sexual pairing to highly social grouping. Although the highly developed social relationships of certain ants, bees, and termites are spectacular, the aggregative behavior of insects that are more independent in habit is equally significant. With these insects, as well as with the more social species, aggregation has a direct bearing on reproduction and survival. Thus, it is a vital factor in their population dynamics.

The particular degree and consistency of the habit for any species may have some selective advantage and therefore is of evolutionary significance also. As indicated by recent work of Wellington (1957), differences in the behavioral responses of individuals in a population can result in different aggregative patterns characteristic of subtypes in the population. Recognition of these subtypes can be helpful in diagnosing population trends. Their proportions indicate both the ecological and genetic structure of the population.

Insects are interesting and challenging subjects for the study of aggregation because of their distinct lifestages and the generally well-defined behavioral activities associated with each lifestage. Owing to differences in the primary activities of the mobile forms—dispersal, feeding, mating, egg-laying, and so forth—the spatial distribution of individuals often changes markedly. This is particularly true of holometabolous species, where a complete change in environment may be involved in successive stages. Moreover, significant changes in spatial distribution sometimes occur within stages.

It is clear that the kind and degree of aggregation must be assessed throughout the entire lifecycle if the ecology of an insect is to be thoroughly investigated and understood. In terms of a life table, the factor of aggregation should be accounted for at each sampling and recording along with the population counts. A measure of aggregation that is biologically sound and statistically valid is essential for the complete analysis of ecological data. It is needed even to develop a reliable method of sampling.

A MEASURE OF AGGREGATION.—The occurrence of an insect in natural units of its habitat usually is best expressed as a frequency series, with the number of sampled units containing 0, 1, 2, $\cdots$, n individuals tabulated in order. Whether the observed frequency distribution is

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random or nonrandom is determined by statistical comparison (usually by means of a Chi-square test) of the observed counts with expected values derived from a theoretical series having known characteristics. The distributions most commonly assumed or determined to be fitted by insect counts are the normal, Poisson, positive binomial, logarithmic, lognormal, Neyman type A, and negative binomial. The first three are random, the others are nonrandom. Empirically and mathematically derived non-random series of the aggregated type are commonly termed "contagious." The inappropriateness of this term and some basic assumptions and relations of random and nonrandom distributions have been discussed by Waters & Henson (1959) with particular reference to forest insects.

Logically, a theoretical distribution that can arise from more than one model or hypothesis should be most useful in assessing the complex phenomenon called aggregation. The negative binomial can be derived from at least five mathematical and biological models (Anscombe 1950, Bliss & Calhoun 1954). Consequently, it has proved applicable to a wide diversity of biological data, particularly for the analysis of insect counts (Anscombe 1949, Wadley 1950, Evans 1953). Its mathematical features are well described (Fisher 1941, Anscombe 1950), and workable methods of computing its statistics have been developed (Bliss & Fisher 1953, Bliss & Owen 1958).

The negative binomial series is characterized by or reflects a variation in the expectation of counts in the observational units, this expectation varying in a distribution proportional to that of Chi-square. It has two parameters, the mean m and an exponent k, which is a measure of dispersion or aggregation. Successive terms of the distribution are obtained by expansion of the expression $(q-p)^{-k}$, where $p=m/k$ and $q=1+p$. Plotting of these terms (the expected probabilities or numbers of units with 0, 1, 2, $\cdots$, n individuals) gives a unimodal curve that is more skewed, with a smaller proportion of zero counts, for a given mean and variance than most other aggregated distributions. The negative binomial is related mathematically to the Poisson and logarithmic series. With increasing randomness, the variance of the distribution approaches the mean, $k\rightarrow\infty$, and the Poisson is obtained as a limit. In the other direction, $k\rightarrow 0$ as aggregation increases, and if only the units with insects are included, the logarithmic series results (Fisher *et al.* 1943).

Thus, the parameter k is a valid measure of aggregation. Its values can range from zero, where aggregation is extreme, to infinity, which defines a purely random distribution of counts. In actual practice, any large value of k indicates an approach toward randomness. For any insect, there will be some field counts which apparently fit both the negative binomial and Poisson distributions. At least, the conventional Chi-square test will show no significant departure from either. In regard to quantifying aggregation, this is not important. The value of k indicates the relative degree of aggregation for the conditions involved. The utility of k as a measure of aggregation is enhanced by ease of computation and also by the fact that the sampling errors pertinent to estimates of it can be calculated readily (Anscombe 1950, Bliss & Owen 1958). The details of these calculations have no place here, but I would like to show how k works by reference to both hypothetical and natural populations of insects.

A HYPOTHETICAL SEQUENCE.—In figure 1 are shown four hypothetical finite populations, comprising 25 units each. In each case, the sample total is fixed at 100 insects. Therefore, the examples are identical except for the distribution of the counts in the 25 units.

Example 1 (see figure) shows a random distribution. The variance of the counts, 4.58, is very nearly equal to the mean of 4—the difference between them is accounted for by the chances of sampling. The calculated estimate of k is 27.59. This is far from infinity but indicates an approximation of randomness (a test of fit with Poisson expectation gives $X^2=27.50$ with 24 degrees of freedom, $P\doteq 0.28$).

Example 2 is an idealized aggregated distribution in which 10 of the units contain 10 insects each, and the remaining 15 units have zero counts. Here, the variance is 25.00, and the calculated k is 0.16.

Example 3 shows a more concentrated aggregative pattern, with 20 insects in each of 5 units and none in the other 20 units. The variance, 66.67, is much larger in this case, and the k is reduced to 0.05.

Example 4 is the extreme case where all 100 insects are found in one unit. The variance of this sample is 400.00, and the k is an infinitesimal 0.006.

An excess of the variance over the mean is a characteristic of all aggregated frequency distributions, including the negative binomial.

EXAMPLES FROM NATURAL POPULATIONS.—Table 1 lists some representative k values for several forest insects at different population levels. Most of these were derived initially in connection with sampling studies. The stages covered are those generally sampled in surveys for appraisal or predictive purposes. The list is incomplete; it is intended primarily to demonstrate the characteristic range in values obtained from field counts. Yet even this preliminary stratification by population level, coupled with some knowledge of the insects involved, provides a basis for interpretation (or speculation). It strongly indicates certain lines for further research.

For example, there is an apparent relationship between k and the population density of spruce budworm (*Choristoneura fumiferana* (Clem.)) larvae on twig units of balsam fir. The lower k values for low populations reflect an initial aggregative tendency in new and/or light infestations and retention of the characteristic as long as suitable hibernating quarters and sufficient food are available and larval movement is minimized.

The aggregative effect may be due to the initial nonrandom deposition of egg masses (note the significant k values for egg masses in table 1), the issuance of the larvae from masses rather than singly, or the movement to and settling of larvae on twigs in certain zones or parts of the tree. But most probably it is caused by the combination of these and perhaps other factors. At high population levels, with greater foliage depletion and increased exposure, competition, and movement, the aggregative effect diminishes, and an approach to randomness appears (indicated by high k values).

Many insect parasites change their searching behavior after they have found and attacked a host. In view of this, it should be interesting to determine the effect of

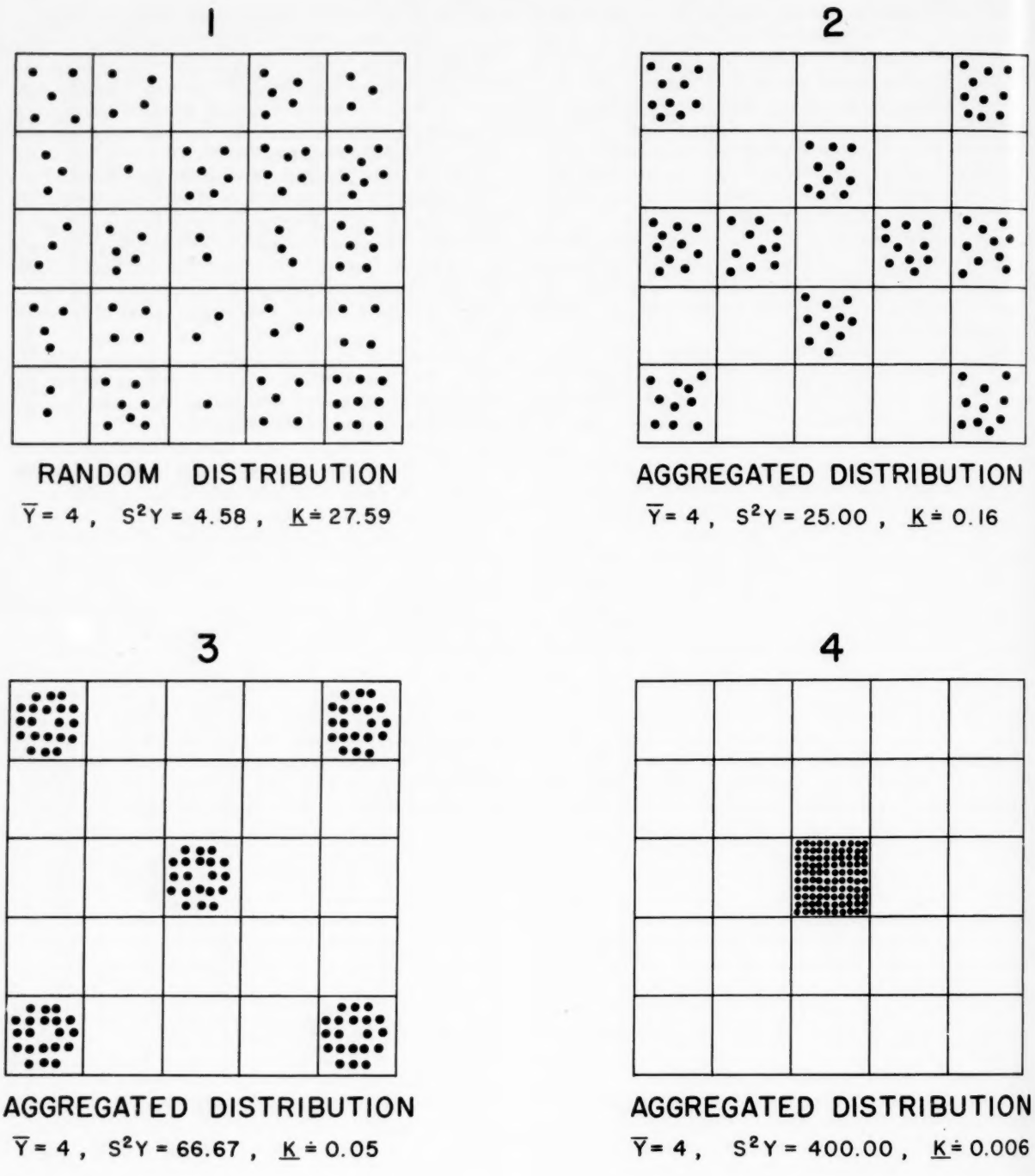
1
RANDOM DISTRIBUTION
$\bar{Y} = 4$, $S^2Y = 4.58$, $\underline{K} \doteq 27.59$
2
AGGREGATED DISTRIBUTION
$\bar{Y} = 4$, $S^2Y = 25.00$, $\underline{K} \doteq 0.16$
3
AGGREGATED DISTRIBUTION
$\bar{Y} = 4$, $S^2Y = 66.67$, $\underline{K} \doteq 0.05$
4
AGGREGATED DISTRIBUTION
$\bar{Y} = 4$, $S^2Y = 400.00$, $\underline{K} \doteq 0.006$
SAMPLE SIZE = 25
NO. OF INSECTS = 100

FIG. 1.—Four hypothetical insect populations differing only in degree of aggregation and resultant k values.

differences in spatial distribution of budworms on the degree of parasitism independent of the influence of host-population density. The nature of the distribution of hosts is undoubtedly a major factor unaccounted for quantitatively in most biological control studies. That this factor can significantly affect the degree of parasitization has been shown experimentally by Burnett (1958).

Table 1.—Representative k values for several forest insects at different population densities.

STAGE	SAMPLING UNIT	POPULATION DENSITY	k RANGE
	Spruce budworm		
Larva (4th–6th instars)	15-inch balsam fir twig	Low	1– 5
		Medium	3–10
		High	6–50
Egg (egg masses)	15-inch balsam fir twig	Low-high	0.1 –10
	Nantucket pine moth		
Larva and pupa	Tip shoot of loblolly pine	Low	∞
		Medium	0.01– 2
		High	1–12
Larva and pupa	Whorl of loblolly pine	Low	∞
		High	1– 2
	Red-pine sawfly (*Neodiprion nanulus* Schedl)		
Egg (egg-infested needles)	15-inch red pine twig	Low-high	0.1 –15
Egg (egg-infested needles)	6-inch tip of red pine	Low-high	0.1 –15
	Forest tent caterpillar (*Malacosoma disstria* Hbn.)		
Egg (egg masses)	Five 30-inch aspen twigs	Low	0.2 –3
		Medium-high	0.5 –5

At low population levels, the larvae and pupae of the Nantucket pine moth, *Rhyacionia frustrana* (Comst.), are distributed in the new buds and shoots of loblolly pine in a random manner. Interestingly, the insect becomes aggregated to a significant degree in medium populations and tends again toward randomness as the population increases. The random effect at low density is due to the very sparsity of the population and consequent low expectation of occurrence of the insect in individual tips. The approach toward randomness at high levels is indicative probably of the saturation level of the population.

DISCUSSION.—As emphasized by Skellam (1952) and Bliss (1958), frequency distributions and pertinent parameters such as the negative binomial k should be used only as descriptions of what is observed and not as analytical tools *per se* in ecological studies. At least, one must be extremely cautious in interpreting the fit of data to theoretical distributions. The quantitative expression of aggregation through a frequency series may reflect a real aggregative tendency, or it may be a statistical artifact.

In brief, the method of sampling and inadvertent compounding of independent random distributions can result in an aggregated distribution. An example of this with the larvae of European chafer, *Amphimallon majalis* (Raz.), in soil samples is described by Bliss (1958). The truly biological bases of aggregation are (1) responses to physical factors of the environment such as light, temperature, and humidity; (2) responses to the host plant, or parts thereof; (3) reproduction behavior (*e.g.*, issuance from egg masses or clumped eggs with limited movement of subsequent stages, clustering of progeny about parents); (4) mutual attraction with other individuals of the same species (*e.g.*, "gregariousness," mating behavior); and (5) interactions with other organisms (*e.g.*, differential elimination of exposed host insects by parasites or predators) (Waters 1958). Examples of these effects are abundant in the literature.

There is a continuous interplay of the biological factors, and the effective or dominant elements change in time and place. The function of any measure of aggregation is to place a quantitative value on the instantaneous resultant of these factors. However, first it is required that the statistical and biological components be distinguished. A sound knowledge of the insect involved is needed for this. Examples of the analyses concerned and the interpretations made possible for several forest insects are given by Waters & Henson (1959).

The use of the negative binomial parameter k as a measure of aggregation has certain limitations and still poses some problems. It is a relative value, not an absolute measure. But any measure of aggregation is dependent in part on the sampling unit chosen, and it must be relative at least to whatever extent the unit itself deviates from an absolute basis of population measurement. Estimates of k from field counts can be obtained by several methods. The efficiency and precision of the formulas used will depend on the character of the counts involved. However, the relative efficiencies of the methods for a variety of conditions are given by Anscombe (1949, 1950). For all cases, the maximum likelihood solution provides a fully efficient estimate of k, but it is practicable only when the counts in any one unit do not exceed 20 or 30 (Bliss & Fisher 1953).

The reliability of k as a measure of aggregation can be much improved by the segregation and analysis of data by population levels and other meaningful strata. This phase of the problem needs further investigation. Also, studies thus far with forest insects indicate that it is advisable to use the smallest feasible natural unit for sampling—*i.e.*, a leaf—and to supplement this with counts on one or more larger units. The analyses will be most informative if the larger units actually contain the smaller and the counts are taken simultaneously.

Much more study is needed on the problem of quantifying aggregation. It may prove to be as important a variate in ecological studies, particularly of population dynamics, as population density itself.

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The Relationship Between Density and Mortality of Flour Beetles Exposed to Insecticide-Treated Surfaces[1]

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ABSTRACT

When high densities (three or more individuals per sq. cm.) of flour beetles, *Tribolium confusum* Duv. and *Tl castaneum* (Hbst.) were exposed to DDT or pyrethrins-treated surfaces in closed petri dishes the resulting mortality was much higher than with low densities (one individual or less per sq. cm.) of beetles. The mortality increase was progressive and did not show an abrupt change over the range of densities studied. The results were obtained from surfaces containing an excess of the insecticide in relation to the amount picked up. The differences in mortality did not occur in uncovered dishes or when the beetles were exposed to a thiocyanate insecticide, Lethane 384® (2-(2-butoxyethoxy)ethyl thiocyanate).

The causes producing such differences in mortality would appear to be associated with some volatile material. Tests of the effects of the secreted quinone compounds and of accumulated carbon dioxide were negative. A critical level of oxygen need is probably reached. This probability is indicated based upon previous experiments in other laboratories that have shown a large increase in oxygen consumption following DDT or pyrethrins exposure. Conversely, a thiocyanate that depresses oxygen consumption did not produce greater mortality with a greater density of beetles. Open dishes would also permit an exchange of air and the differences in mortality did not occur under these conditions.

Other possible influencing factors were studied but did not produce a positive correlation with the differences in mortality. These factors included the possible effect of rate of movement and pickup of the insecticide. Rate of movement was greater with a greater density of beetles, which occurred whether the dishes were covered or uncovered. No marked difference occurred in pickup.

This study is concerned with the differences in mortality which occur when different numbers of individuals of two species of flour beetles are exposed to an insecticide, DDT, pyrethrins, or Lethane 384,® (2-(2-butoxyethoxy)ethyl thiocyanate), applied on the surface of filter papers.

Numerous studies using standardized insecticide tests have dealt with irregularities in the response of insects. The relative importance of the effect of certain physical and biological factors on results have been discussed by Sun (1950) with comparisons involving two or more insecticides. Recently, Busvine has presented a critical review of testing techniques (1957). Many test procedures stress the importance of using a constant number of individuals. One factor seldom given serious attention is the problem of using a contrasting number of individuals in a given test. Three such studies have been made with mosquito larvae as reported by Krusé *et al.* (1952), Schmidt & Weidhaas (1958), and Wattal & Cutkomp (1959). These studies indicated that by altering the number of larvae being tested in a given vessel, one could obtain different responses to DDT as measured by mortality. The present study involves similar considerations with flour beetles as test insects. The method of testing was necessarily different. The results obtained were not anticipated and some effort has been made to provide an explanation of them.

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[3] The assistance of Dr. M. Govindan and R. L. Tripathi was most helpful and is greatly appreciated.

METHODS AND MATERIALS.—Adults of the confused flour beetles, *Tribolium confusum* Duv., were used in most experiments and several comparative tests involved the red flour beetle, *Tribolium castaneum* (Hbst.). Both species were reared from established cultures using a good mix of branless wheat flour and 5% dried brewer's yeast. Rearing procedures followed those of Shepard (1943) and employed temperatures of 25° to 27° C. Eggs were collected at least once a week to provide adults of known similar ages by sifting the food medium.

The source of pyrethrins was a fresh solution of pyrethrum (Pyrocide 175) diluted in the refined kerosene (Deobase). A technical grade of DDT dissolved in acetone was used for liquid application of filter papers. Later experiments were carried out with DDT in Deobase. Less extensive tests were conducted with dusts of both insecticides. A pyrethrum dust concentrate (Pyrocide having a 40% pyrethrins content) diluted with an inert diluent, Neutralite, was used as well as dusts containing 1.0 and 1.5% technical DDT diluted with Pyrax from a 50% DDT concentrate. One set of experiments was carried out with Lethane 384.

PROCEDURE.—The *Tribolium* adults were exposed for definite periods to insecticide-treated filter papers fitted tightly into petri dishes having a diameter of 9 cm. *Tribolium* cannot be tested fairly on glass surfaces because of their inability to walk on such smooth surfaces. The treatment and posttreatment periods were carried out in the dark at a temperature of 25° C. At the conclusion of exposure to the insecticide, the insects were transferred to the food medium and kept in 2-oz. seamless tins. To avoid crowding during recovery the *Tribolium* did not exceed 150 when kept in the tins. They were reduced to 25 in many recovery tins to insure uniform recovery conditions. Most counts for mortality were made 7 days after the insects had been transferred to the tins.

The pyrethrins in Deobase were applied from a measuring pipette to semicrepe filter paper. The rate of application was 3.44 mg. of pyrethrins in 0.5 ml. liquid applied to a filter paper area of 63.9 square cm., resulting in approximately 50 mg. pyrethrins per square ft. A technical grade of DDT in solution in acetone or in Deobase was applied to the filter paper in a similar manner. Six-tenths of a ml. was applied to the filter paper, resulting in 9.3 mg. DDT per sq. ft.

All dust applications were applied uniformly by means of a precision vacuum type duster adapted from the design of Farrar *et al.* (1949). One-half gram of dust was applied each time and released by pulling a vacuum of 10″ of mercury and permitting air to rush in by breaking the partial vacuum. *T. confusum* was the only species used in the dusting experiments.

Since all the early experiments were carried out in covered petri dishes and gave rather unexpected results, comparative tests were made in covered dishes and in covered dishes in which activated carbon particles were enclosed in a small envelope in the cover of the petri dish. The carbon was used to permit sorption of vapors without contacting the beetles or the insecticide-treated surface. When the effect of compounds secreted by *Tribolium* sp. was studied the chemicals were impregnated in cellulose-type pieces of sponge that were fastened inside the covers. In one experiment the benzoquinones were added to the

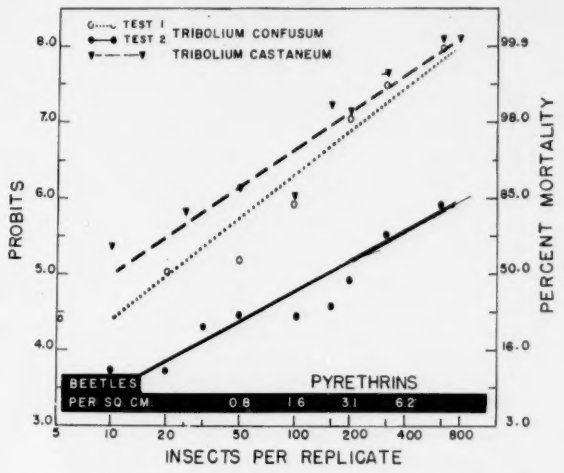

FIG. 1.—A comparison of the mortality resulting from a residual application of pyrethrins solutions when different numbers of *Tribolium confusum* and *T. castaneum* adults were exposed in covered petri dishes. Beetles were between 3 and 4 weeks old. Exposure time was 24 hours. Pyrethrins were applied at the rate of 50 mg. per sq. ft.

pyrethrins and DDT-treated papers. A test to study the results when carbon dioxide was removed involved the use of saturated barium hydroxide placed in a small cup in the center of the treated filter paper. This preparation served to absorb the carbon dioxide output of the beetles without the possibility of their direct contact, and without contaminating the treated paper.

The determination of pickup of DDT by beetles was made by using radioactive DDT having double-labeled C^{14} on the phenyl groups.[4] The labeled DDT was applied in solution to sheets of tissue paper. Adult beetles were exposed as in other experiments and at selected times (usually 4 hours) were removed from the papers. Such treated individuals were quick frozen and subsequently the DDT was washed off beetles using small quantities of an organic solvent, such as chemically pure benzene. Counts from planchets were made in a proportional nuclear counter to determine the comparative pickup of different treatments.

RESULTS.—The results are presented in four parts: (1) the effect of pyrethrins, (2) the effect of DDT, (3) the effect of Lethane 384, and (4) possible causes of anomalous results.

(1) *Study with Pyrethrins.*—A series of four different tests was made with *T. confusum* adults exposed to filter papers treated with pyrethrins solution in Deobase. The individuals ranged from 5 to 600 per petri dish. The results of two such tests are given in figure 1, as well as a test with *Tribolium castaneum*. The trend of increased per cent mortality with increased numbers of insects was quite consistent, ranging from 27% when 5 were used to 100% when 600 specimens were included in one experiment. A series of three replications using *T. castaneum* indicated a similar trend. The per cent mortality was 63, 84, and 100 with numbers of 10, 100, and 600 beetles, re-

[4] Preparation obtained from Dr. Paul Dahm, Iowa State University, Ames. Synthesis with a specific activity of 0.75 millicuries per millimole (2.13μc./mg.) carried out by Tracerlab, Inc., Boston, Massachusetts.

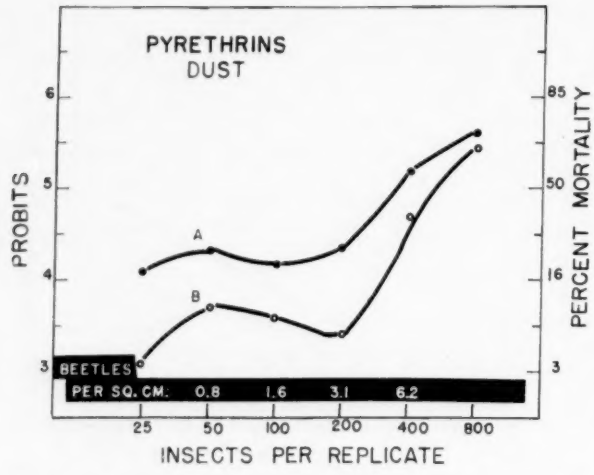

FIG. 2.—A comparison of the mortality resulting from dust applications of pyrethrins when different numbers of *Tribolium confusum* were exposed in covered petri dishes for 24 hours. Beetles were 29 and 30 days old. Curve A shows the result when beetles were dusted directly and Curve B when the dusts were applied to the filter papers before introducing the beetles. Pyrethrins were applied at the rate of 40 mg. per sq. ft.

spectively. This species was somewhat more susceptible to pyrethrins.

A second study with pyrethrins was carried out with dust applications to the insects alone and a comparative application made to the walking surface for the beetles. The numbers of *T. confusum* used per replicate was 25 for the smaller numbers and 800 and 1,500 for the large numbers. When greater numbers were used the per cent mortality was higher. This result was in accord with the first involving pyrethrins in oil. Certain differences were obtained, however, that were not apparent in the earlier studies made with pyrethrins in an oil base (fig. 2). For example, when insect mortality versus numbers of insects was plotted on a probit-log basis a straight regression line resulted for other tests, but a bimodal curve was indicated by the results from dusting. Significant differences in mortality occurred only at high densities, such as 400 or 800 beetles per dish. Low mortality occurred with small numbers.

A greater susceptibility was noted with 16-day-old adults as compared with the 29- and 30-day-old lots, but otherwise the trend remained the same for the different age groups.

(2) *Study with DDT.*—The toxicity of DDT to *Tribolium confusum* and *T. castaneum* is expressed much much more slowly when compared with pyrethrins. It was of interest to see if the pattern of greater per cent mortality, as concerned with increased density of beetles, would occur with DDT as it did with pyrethrins. Four different tests were carried out using 0.01 mg. DDT per sq. cm. of filter paper. The adult beetles per petri dish ranged from 5 through 800 and involved flour beetles of selected age levels. As in the experiment with pyrethrins, a decided increase in mortality of *T. confusum* from DDT exposure occurred, ranging from 20% with 10 beetles to 69.6% with 600 beetles (fig. 3). The increased mortality was not quite so striking as with pyrethrins. Furthermore, there was some indication that a linear relationship did not exist, since when the beetles varied between 150 and 200 the per cent mortality was in some cases as low or lower than in the 50- or 100-beetles density groups. When the density of beetles was 400 or greater, however, the per cent mortality was considerably higher (fig. 3). The closely related beetle, *T. castaneum*, responded in a similar manner to DDT, the per cent mortality values being 4, 46, and 80 when the numbers of beetles were 25, 200 and 800, respectively (fig. 3).

Experiments with dusts of DDT were inconclusive. Dusts were applied in two ways. In one case the dust was applied directly on the filter paper before the beetles were put in the dishes, and in the second case the beetles were dusted directly. These tests, with a limited number of replications, indicated no significant differences in *Tribolium* mortality when 25, 50, 100, 200, 300, and 800 beetles were used. This was the only experiment that failed to show significant increases in per cent mortality accompanying increases in numbers of beetles per petri dish.

(3) *Study with Lethane 384.*—This thiocyanate was purposely chosen because of information indicating that it affected the respiratory rate of insects differently than pyrethrins and DDT (Harvey & Brown 1951). The interpretation of results is presented in the discussion.

Lethane 384 was used at a 5% concentration on filter papers and a comparison was made with two densities, 25 and 400 adults of *Tribolium confusum*. The results do not correspond to those obtained with DDT and pyrethrins. Mortality showed no increase with greater density of beetles. Actually, the trend is in the opposite direction. Results are presented in table 1.

(4) *Possible Causes of Anomolous Results. The Effects of Vapors.*—Since unexpected results occurred when the exposure period was spent in covered petri dishes, the importance of vapor toxicity needed to be checked. Conceivably vapors from carbon dioxide or the secretory

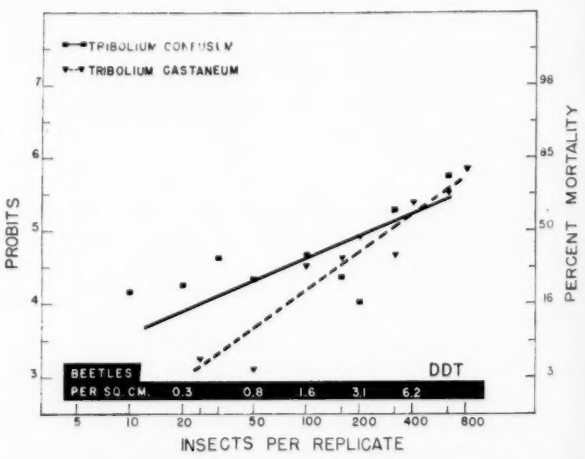

FIG. 3.—A comparison of the mortality resulting from a residual application of DDT in acetone when different numbers of *Tribolium confusum* and *T. castaneum* were exposed in covered petri dishes. *Tribolium confusum* adults were 26 days old, *T. castaneum* of mixed ages. Exposure time was 24 hours. DDT was applied at the rate of 9.3 mg. per sq. ft.

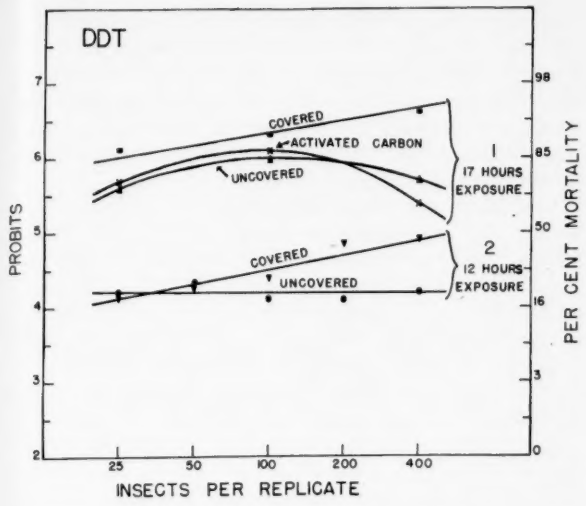

FIG. 4.—A comparison of the mortality resulting from DDT when different numbers of *Tribolium confusum* adults exposed in uncovered petri dishes, covered petri dishes, and covered dishes containing a small packet of activated charcoal in the cover. The charcoal was used to sorb vapors within the dish. DDT was applied to the filter papers at the rate of 9.3 mg. per sq. ft.

material of *Tribolium* (benzoquinones) could be involved. The vapors from pyrethrins should not have been a factor, since DDT gave a similar effect. The importance of vapor was recognized after carrying out an experiment in which the exposure was made in open dishes and in dishes containing activated carbon in contrast to covered dishes. The numbers of beetles were varied from 25 to 400 per dish. The results in figure 4 indicate that vapors were important, since no significant difference occurred in mortality owing to number of insects when the dishes were uncovered or when covered dishes contained activated carbon.

Studies were conducted with the compounds secreted by flour beetles. Greater quantities may be secreted under stress. These experiments included the additions of the quinone compounds, 2-ethyl-1,4-benzoquinone and 2-methyl-1,4-benzoquinone, during beetle exposure to the insecticide. These compounds represent more than 95% of the odorous secretion present in *Tribolium* (Loconti & Roth 1953).[5] Results of the addition of these compounds directly to the insecticide-treated surface or in cellulose sponges in the covers of the dishes are presented in table 2. No indication of increased mortality attributable to the addition of these compounds was evident.

Table 1.—Comparative mortalities of beetles exposed to a residual treatment of Lethane 384.[a]

No. of Beetles per Dish	Total Number of Beetles per Treatment	Per Cent Mortality
25	76	64.5
400	1,187	39.4

[a] Contrasting numbers of *Tribolium confusum* (4-week-old adults) were exposed to a residue of about 30 mg. Lethane 384 per sq. ft. for 24 hours. Three replications are included.

Table 2.—Results of tests to determine if the addition of two quinone compounds would change mortality of *Tribolium confusum* exposed to 50 mg. of pyrethrins per sq. foot of filter paper in covered petri dishes.[a]

Treatment	Experiment A		Experiment B	
	Total No. of Insects	Per Cent Mortality	Total No. of Insects	Per Cent Mortality
Pyrethrins	124	24.2[b]	100	79
Pyrethrins+ ethylquinone	124	17.7	100	71
Pyrethrins+ toluquinone	125	25.7	100	88
Control (solvent only)	125	1.6	100	11

[a] Experiment A.—Two milligrams of quinone crystals were applied to filter paper surface (five replications). Experiment B.—Five milligrams of quinone crystals dissolved in acetone were deposited in cellulose sponges attached to dish covers (four replications).

[b] No significant differences between treatments; treatments in experiment A not comparable to treatments in experiment B, because a different culture of insects was involved.

The vapor effect of beetle secretions was tested also by introducing treated beetles into a small central container or well in the petri dish. These beetles have been exposed to pyrethrins for 15 minutes and were not capable of locomotion. These affected beetles, equal in number to a high density of beetles under test, emitted vapors of their secretions which could influence the mortality. Table 3 shows some increase in mortality when such affected beetles were added, but this effect could not match or equal the mortality in treatments where 250 beetles were used per dish.

The possibility that carbon dioxide was a factor was checked also, by using saturated barium hydroxide as the absorbent. Again (table 4) no indication was evident that the carbon dioxide contributed to the increased mortality when greater numbers of beetles were present. Actually, the addition of barium hydroxide resulted in an increase in mortality, whereas if carbon dioxide accumulation had been involved a decrease should have occurred with its removal.

Table 3.—Results of testing the effect of adding pyrethrins-affected beetles in a small center glass container while other *Tribolium* were exposed to pyrethrins-treated filter paper in covered petri dishes for 18 hours.[a]

Treatment	Modification	Number Insects per Dish	Per Cent Mortality
Pyrethrins	None	25	17
	225 pyrethrins-affected beetles in center well	25	35
	None	250	58
Untreated	None	25	1

[a] Four replications used per treatment and mortality taken at 7 days.

ACTIVITY OF THE INSECTS AS A FACTOR IN MORTALITY DIFFERENCES.—The possibility of increased activity of

[5] The authors gratefully acknowledge the receipt of the quinone compounds from L. M. Roth, Biology Branch, Hq. Quartermaster Research and Development Command, Natick, Massachusetts.

Table 4.—Results of test to determine possible effect of accumulation of carbon dioxide in dishes on mortality of *Tribolium confusum* exposed 24 hours to 50 mg. of pyrethrins per sq. foot on filter paper in covered petri dishes.[a]

Treatment	No. Insects		Per Cent Mortality
	Per Dish	Total	
Pyrethrins	25	72	25.0
Pyrethrins+ $Ba(OH)_2$	25	73	89.0
Pyrethrins	400	1,323	99.1
Pyrethrins+ $Ba(HO)_2$	400	1,342	100
Control (Solvent only)	25	76	0

[a] Three replicates were used per treatment. Saturated Ba $(OH)_2$ was used in an open container and was not accessible to the insects and did not touch insecticide.

the beetles as a result of exposure to the film of insecticide was checked. Although vapor effects appear to predominate, increased activity could result in increased uptake or pickup of the insecticide. Motion pictures were taken to obtain results pertaining to the beetle movement.

A 16-mm. movie camera (Maurer) was used with a film rate of 24 frames per second. Fast films were employed to make possible the photographing of the activity of the beetles with rather low intensity lighting. The most useful times to photograph insect movements were determined from previous observations of their activity on pyrethrins and DDT-treated surfaces.

Pyrethrins produce rapid responses. Since knockdown may be nearly complete in 7 to 12 minutes, the times for filming were 1, 3, 5, and 7.5 minutes after beetles were exposed to the treated surface. Insect movement was extremely slow after this period. In the case of DDT the times for filming were 15, 30, 60, 120, and 180 minutes after exposure to the treated surface. Movement had declined considerably after 3 hours. Untreated controls were filmed at the same time periods as the insecticide-treated beetles.

Counts of movement were made by projection producing enlarged images on a screen. Individual beetles were marked with white paint but in practice it was found the movement of single unmarked beetles could be followed. By dividing the projection screen into quadrants containing 2-inch squares the progress of a single beetle could be determined. Beetles were selected at random for counting. The results of the studies of movements with pyrethrins treatment and untreated are presented in figure 5 and the results from the DDT experiments are in figure 6. The rate of movement of individuals is greater with greater densities of insects, but this greater rate of movement occurred both in open or covered petri dishes. The closure of the test dishes thus did not produce any significant difference in rate of movement. It will be noted that insect activity showed a general decline over the time period studied. Untreated ones also showed some decline.

The information on movement rate is of interest on a comparative basis. The maximum speeds of untreated beetles in open dishes was 7.8 mm. per second (among 100 beetles) and 8.9 mm. per second (among 400 beetles) in covered dishes (fig. 5). The temperature was 21° C. These movements were measured after the beetles were on the surface for 2 minutes. When 25 beetles were in a dish the rates of movement were essentially the same whether covered or uncovered. They were 5.5 (covered) and 5.6

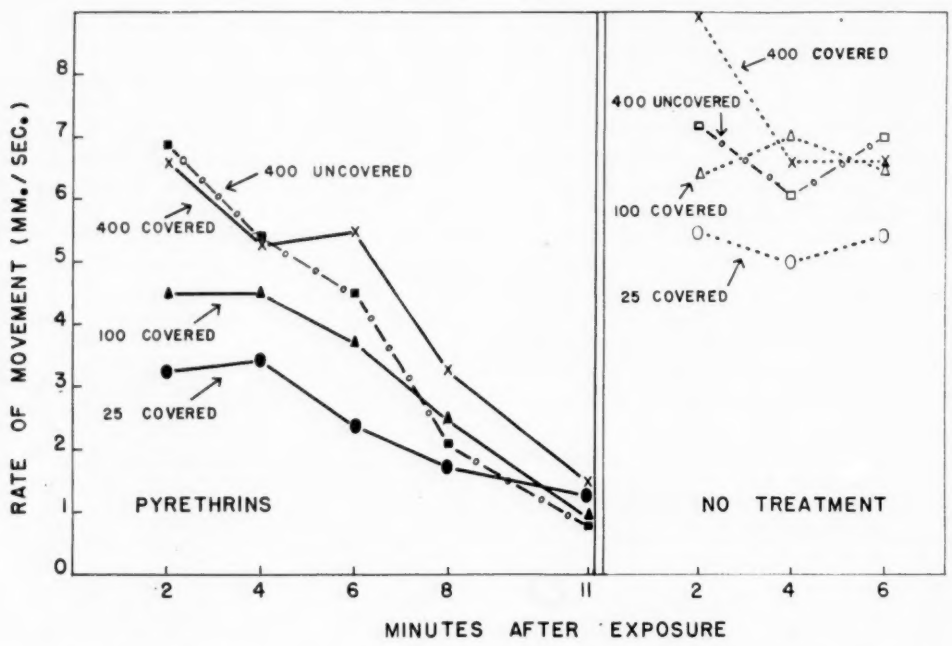

FIG. 5.—A comparison of the rate of movement of individual beetles within groups of 25, 100, and 400 beetles. The movement of the pyrethrins-treated groups is charted on the left and untreated are on the right. Most results are given from covered dishes, with one comparison shown for 400 beetles per covered and uncovered dishes.

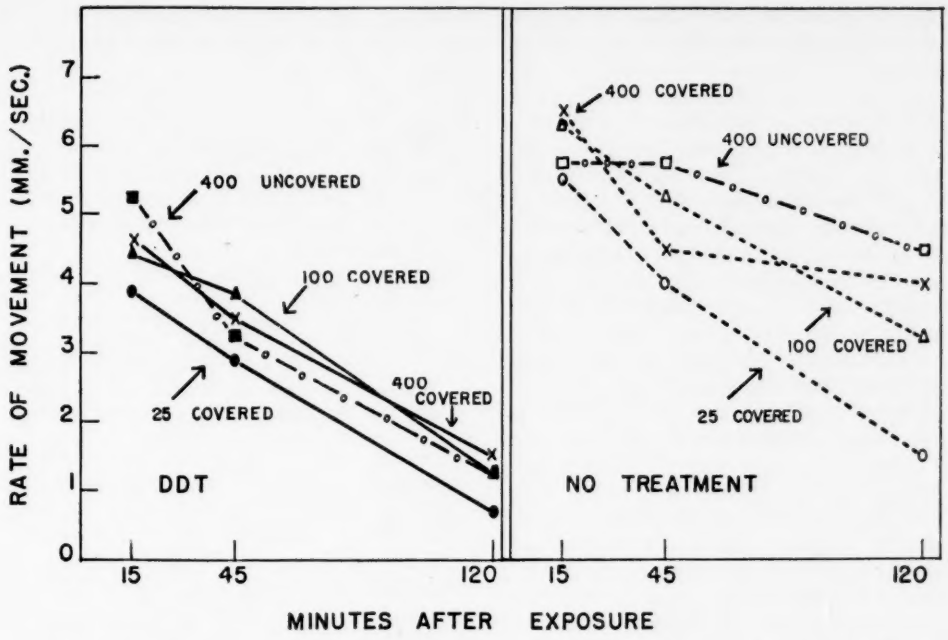

FIG. 6.—A comparison of the rate of movement of individual beetles within groups of 25, 100, and 400 beetles. The movement of the DDT-treated groups is charted on the left and untreated on the right. Most results are given from covered dishes, with one comparison shown for 400 beetles per covered and uncovered dishes.

mm. (uncovered) per second. The rates had dropped to 1.5 (covered) and 4.0 mm. (uncovered) after the beetles had been on the surfaces for 2 hours. This count is taken when the beetles are out of their normal environment of flour, of course. A value of 5 mm. per second is approximately one-third the speed attributed to the ant, *Lasius niger* Emery, and appears to be the slowest of those insects where records are available (McConnell & Richards 1955).

PICKUP OF DDT.—The quantity of DDT picked up, as measured with radioactive-labeled DDT, was determined for different densities of *Tribolium confusum* exposed on tissue papers in covered and uncovered petri dishes. The results are presented in table 5. The results show a trend indicating somewhat greater pickup of DDT (as radioactive carbon) when higher densities of beetles are used. However, the effect which might be due to covering dishes was not apparent in pickup measurements. Thus, any difference in quantity picked up does not correspond to the mortality data. In these experiments 400 beetles (6.2 beetles per sq. cm.) and 600 beetles (9.4 beetles per sq. cm.) were used to create the high density.

DISCUSSION.—High densities of flour beetles exposed to pyrethrins or DDT-treated filter papers are accompanied by high mortality when covers are left on the exposure dishes. The amount of available insecticide per dish (2 mg. in the case of DDT) was much in excess of total amounts picked up. The mortality differences are very significant when low densities of 5 to 25 beetles per petri dish (0.08 to 0.4 beetles per square centimeter) are compared with high densities of more than 200 beetles or more (3 to 12 beetles per sq. cm.). The increase appears to approach a logarithmic relationship rather than an arithmetical one, although in some instances intermediate densities (1.5 to 2.5 beetles per sq. cm.) are not associated with significantly higher mortalities.

Since the density of beetles does not have any significant effect on mortality in open dishes or when activated charcoal is used in covers to sorb vapors, the cause of the mortality differences appears to be due to volatile materials. The effect from vapors could be direct or indirect. The secretion of quinone-type compounds that have been shown to have biological activity (Loconti & Roth 1953) may be sufficient to account for the increased mortality of beetles when higher densities are tested, but the addition of the two most prominent of these chemicals to the experimental dishes did not increase the mortality. Thus,

Table 5.—The quantity of C^{14} found per beetle using C^{14} labeled DDT from samples of 15 beetles from each treatment replicate.[a]

NUMBER OF BEETLES			
Per Dish	Per sq. cm.	STATUS OF PETRI DISHES	APPROXIMATE MICROGRAMS DDT PICKED UP/BEETLE
25	0.4	Covered	0.0283 ± [b]0.0109[c]
			.0210 ± 0.0022
25	0.4	Uncovered	.0154 ± .0090
400	6.2	Covered	.0324 ± .0178
600	9.4	Covered	.0275 ± .0086
400	6.2	Uncovered	.0364 ± .0194

[a] Each microgram of C^{14} labeled DDT produced from 4,333 to 4,460 counts per minute on the proportional counter used. Three replicates used per treatment. Time of exposure to 2 mg. DDT on each filter paper, 5 hours.

[b] ± values are S.E.

[c] The first value given for 25 beetles (0.0283) was determined from the same population of beetles as the value for 600 beetles (0.0275). All other values were determined from another population of beetles taken a few days earlier.

they do not appear to be the likely causes of increased mortality. Accumulation of carbon dioxide does not appear to be involved.

Differences in rate of movement and pickup of insecticide might also be clues to a change in effect. A greater rate of movement was noticed with greater densities of beetles. No significant difference occurred in amount of pickup. However, a comparison in covered and uncovered petri dishes gave no significant differences in rate of movement. The quantity of DDT picked up was somewhat greater in open dishes with a high density of beetles. This result is, of course, opposite to the mortality results where greater kill took place in closed dishes. These results could indicate that rate of movement and pickup are not factors in producing the anomalous mortality results.

The cause or causes of increased mortality of flour beetles when tested in greater density in closed petri dishes appears to be found in indirect evidence. For the present the evidence is based upon information on oxygen uptake or gaseous exchange during respiration. Both DDT and pyrethrins have been shown to produce a marked increase in oxygen consumption in *Tribolium castaneum* (Lord 1950) and in the German cockroach, *Blattella germanica* (L.) (Harvey & Brown 1951). The thiocyanate, Lethane 384, causes an immediate and continuous depression of the oxygen consumption rate (Harvey & Brown 1951). From such evidence it can be deduced that when many beetles are exposed to DDT or pyrethrins in closed petri dishes they use up sufficient oxygen to reach a critical need and thus succumb more readily to the quantity of insecticide they have contacted. The exposure to the thiocyanate, on the other hand, has not caused any critical need for oxygen, and mortality is not increased in covered dishes or when the density of beetles is high. It is hoped that direct evidence to support this explanation can be obtained in the near future.

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The Metabolism of Dimethoate in Cattle[1]

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Abstract

The metabolism of P^{32}-dimethoate was studied following oral and intramuscular administration (10 mg./kg.) to cattle. By both routes high radioactivity was detected in the blood shortly after administration. The radioactivity, representing both total and organosoluble compounds, was observed earlier and dissipated faster in the intramuscular treatment. Chromatographic analyses of blood extracts indicated the presence of both dimethoate and unknown(s), with the latter several times more toxic than the parent compound as determined by enzymic analyses and bioassay.

About 87 to 90% of the oral dose was eliminated in the urine at the end of 24 hours. The same percentage of intramuscular dose was excreted after 9 hours. The major metabolic products were dimethyl phosphate, dimethyl phosphorothioate, and several unknowns. Only 3.7 to 5% of the oral dose and about 1.1% of the intramuscular dose were eliminated in the feces.

Analyses of tissues from an orally treated calf showed only very low levels (0.02 to 0.07 μg./gram) of organoextractable radioactive compounds present in the brain, liver, testes, and lungs.

Dimethoate, known chemically as *O,O*-dimethyl-S-(*N*-methylcarbamoylmethyl) phosphorodithioate, has shown promise as a systemic for the control of first-instar cattle grubs (Hewett *et al.* 1958) when administered either orally or intramuscularly. Studies were conducted to provide information on the absorption, excretion, and metabolism of this compound when administered to cattle at toxicologically safe rates and routes of treatment. Radiotracers, bioassay, enzymic assay, and paper chromatographic methods were employed.

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MATERIALS AND METHODS.—The dosage for all the animals used in this study was 10 mg./kg. Two calves were treated orally by capsule with the compound, and a third was injected intramuscularly with a 50% solution (w/w) in diethyl succinate. The P^{32}-labeled dimethoate (formerly known as Am. Cyanamid 12880) was found to have a purity greater than 98% by paper chromatography, anticholinesterase activity, and infrared analyses. The treatment data are summarized below:

	Calf 1	*Calf 2*	*Calf 3*
Locality	Corvallis, Oregon	Kerrville, Texas	Kerrville, Texas
Animal (all Herefords)	Bull	Steer	Steer
Method of administration	Oral	Oral	Intramuscular
Weight (kg.)	161	186	194
Observable specific activity (c.p.m./gm.)	1.11×10^9	7.35×10^8	5.52×10^8

Following administration of the labeled compound, the animals were held in metabolism cages and provided with food and water. At various intervals samples of blood, urine, and feces were taken for analyses. Two weeks after treatment calf 1 was sacrificed and tissue samples were taken for analyses. The methods for sample collection, preparation, and radioassay have been previously described (Robbins *et al.* 1956, Kaplanis *et al.* 1959).

Total radioactive compounds in the blood were assayed by counting plated samples of whole blood. The organosoluble compounds were determined by extracting whole blood or whole blood diluted with distilled water (1:1) with an equal volume of dichloromethane. When control blood samples to which known quantities of dimethoate had been added were treated in the same manner, 93% of the compound was found to partition into the dichloromethane.

The total radioactive compounds in the urine were determined by assay of plated urine samples. Organosoluble metabolites were determined by extracting urine adjusted to various pH levels with an equal volume of chloroform.

The fecal samples were prepared for radioassay by wet-ashing or homogenization. The organosoluble radioactive compounds were determined by extracting feces homogenates with an equal volume of chloroform.

The total radioactivity in the tissues was determined from plated tissue homogenates prepared by blending 100-gram samples to give a final concentration of 0.4 gram of tissue (wet weight) per milliliter of homogenate. Organosoluble compounds were determined by adding 0.02 to 0.04 ml. of 50% trichloroacetic acid solution per milliliter of homogenate with an equal volume of chloroform. The water-soluble compounds were determined from plated aliquots of the clear aqueous layer after centrifugation. Fat was analyzed by direct extraction in a blender with chloroform. The extracts were dried over sodium sulfate, filtered, made up to a volume, and aliquots plated for radioassay.

Several paper chromatographic systems were used for the analysis of dimethoate and related compounds. The primary ones were: (a) Chloroform-heptane/ethylene glycol. Whatman No. 1 paper coated with a 25% solution of ethylene glycol in acetone was developed with chloroform-heptane (9:1) v/v. (b) Vaseline-*n*-butanol. Whatman No. 1 paper coated with a 3% solution (w/v) of vaseline in chloroform constituted the stationary phase. The developing medium consisted of *n*-butanol saturated with water. (c) Acetonitrile-water. Uncoated Whatman No. 1 paper served as the stationary phase. The developing solvent consisted of either 8.5:1.5 or 8:2 parts of acetonitrile and water.

An 18- to 40-cm. ascending development was used in these systems. The R_f values for dimethoate and related metabolites are shown in table 1. Methods for detection have been described (Robbins *et al.* 1957).

The dichloromethane extracts of the blood of calf 1 were pooled (1 to 6 hours), concentrated, and chromatographed by the chloroform-heptane/ethylene glycol system. The compounds were analyzed for anti-ChE activity with house fly heads as an enzyme source. The extracts from calves 2 and 3 were chromatographed in the vaseline-*n*-butanol system. The 1-cm. strips of the developed chromatogram containing the unknown were extracted three times with 25-ml. aliquots of acetone in a separatory funnel. The extracts were pooled, concentrated, and radioassayed to determine the quantity available for bioassay on stable flies when added to the blood meal.

The RBC ChE activity was determined by the colorimetric whole-blood method (Robbins *et al.* 1958) and the electrometric technique (Radeleff & Woodard 1956).

RESULTS.—The blood data are given in table 2. High levels of radioactivity were detected in the blood of the animals treated by both routes shortly after administration of the labeled material. The observable radioactivity representing both total and organosoluble compounds occurred earlier and dissipated faster in the intramuscular treatment.

Paper chromatographic analyses on the 1- to 6-hour composite dichloromethane extracts from calf 1 showed that about 87% of the radioactivity ran with the R_f of dimethoate and 12% as an unknown(s) (R_f 0.39). Enzymic analyses of the unknown demonstrated an IN_{50} of about 8.2×10^{-7} M against house fly head ChE; that of dimethoate is 3.0×10^{-4} M.

The RBC ChE determinations demonstrated maximum inhibitions of 31, 69, and 42% for calves 1, 2, and 3, respectively. A recovery to 24% at the end of 2 weeks was observed for calf 1.

Analyses of the blood extracts from calves 2 and 3 as determined by the vaseline-*n*-butanol system are shown

Table 1.—R_f values of P^{32}-dimethoate and related compounds as determined by several paper chromatographic systems.

COMPOUND	CHLOROFORM HEPTANE/ ETHYLENE GLYCOL	VASELINE-*n*-BUTANOL	ACETONITRILE: WATER 8.5:1.5	ACETONITRILE: WATER 8:2
Dimethoate	0.72	0.85	0.87	0.93
O,O-dimethyl phosphate	.00	.05	.07	.15
O,O-dimethyl phosphorothioate	.00	.10	.21	.35
O,O-dimethyl phosphorodithioate	.00	.17	.40	.55
Unknown(s) 1[a]	—	—	.28[a] .33	.68
Unknown(s) 2	—	—	.03	.04
Unknown(s) 3	—	—	.52	.79
Unknown(s) 4	—	—	—	.25
Unknown(s) 5	—	—	—	.45

[a] Found to be two metabolites that were difficult to separate.

Table 2.—Radioactive compounds in the blood and RBC ChE activity of calves treated with P^{32}-dimethoate.

	Oral Treatments						Intramuscular Treatments		
	Calf 1			Calf 2			Calf 3		
	Radioactive Compounds (μg.-Equiv./ml.)		Inhibition of RBC ChE Activity (%)	Radioactive Compounds (μg.-Equiv./Ml.)		Inhibition of RBC ChE Activity %[a]	Radioactive Compounds (μg.-Equiv./Ml.)		Inhibition of RBC ChE Activity %
Time	Total	DEC[c]		Total	DEC[c]		Total	DEC[c]	
1 hour	4.26	1.13	—	3.09	0.36	28	10.12	4.91	32
3	6.82	2.30	—	4.34	0.74	17	5.91	1.65	42
6	4.52	0.89	—	5.32	1.01	36	2.26	0.41	16
9	2.34	.18	—	4.35	0.89	42	0.97	.25	2
12	1.22	.05	30	2.80	.68	36	.46	Trace	—
18	0.47	Trace	—	1.83	.39	47	.11	0	11
24	0.29	0	31	1.00	.18	56	.06[b]	0	—
30	—	—	—	0.50	.05	44	.05[b]	0	26
36	.16	0	28	.29	Trace	47	0.05[b]	0	21
48	.09	—	24	.10	0	56	0	0	37
60	—	—	—	.08	0	67	0	0	26
3 days	0.04[b]	—	29	.02	0	67	0	0	26
4	—	—	—	Trace	0	69	0	0	16
5	0.04[b]	—	28	Trace	0	56	0	0	16
6	—	—	—	Trace	0	53	0	0	11
7	.04[b]	—	27	Trace	0	44	0	0	—
10	.04[b]	—	23	—	—	—	0	0	—
14	.03[b]	—	24	—	—	—	—	—	—

[a] Cholinesterase determinations by R. D. Radeleff.
[b] Standard error greater than ±5%.
[c] Dichloromethane-Extractable Compounds.

in table 3. With both treatments the percentage behaving as dimethoate decreased whereas that representing the unknown(s) (R_f 0.64) increased with time.

Bioassay of the unknown on stable flies showed it to be 40 times more toxic than the parent compound (Roberts *et al.* 1958).

Dimethoate is rapidly metabolized, and the degradation products are excreted via the urine (table 4). About 87 to 90% of the dose was accounted for in the urine 24 hours after oral and 9 hours after intramuscular administration. Extraction of urine from calf 1 with an equal volume of chloroform (table 5) demonstrated that not more than 0.2% of the compounds present was extractable from alkaline urine (pH 9). When the urine was adjusted to pH 4 and 0 with concentrated hydrochloric acid, a higher percentage of the radioactive compounds present partitioned into the organic layer.

The results of paper chromatographic analyses of the urine by the acetonitrile-water systems are given in table 6. The major metabolic products were dimethyl phosphate, dimethyl phosphorothioate, and unknown(s) 1. The latter was observed to break down slowly to form unknown(s) 2. The radioactive compounds from the pooled 1- to 18-hour chloroform extracts of acid urine (pH 4 and 0) ran with the R_f of unknown(s) 1. Only traces of unmetabolized dimethoate were found in the urine.

The efficient absorption of this compound is further shown in the lower levels of radioactive compounds found in the feces (table 7). Only 3.7 to 5% of the administered

Table 3.—Percentages of dimethoate and unknown(s) from blood of Hereford calves treated with P^{32}-dimethoate.

	Oral (Calf 2)		Intramuscular (Calf 3)	
Hours	Dimethoate	Unknown	Dimethoate	Unknown
1	93	7	98	2
3	87	13	90	10
6	84	16	51	49
9	84	16	33	67
12	77	23	—	—
18	75	25	—	—
24	62	38	—	—
30	Trace	Trace	—	—

Table 4.—Radioactive compounds in the urine of three calves treated with P^{32}-dimethoate.

	Oral Treatment				Intramuscular Treatment	
	Calf 1		Calf 2		Calf 3	
Time	Mg.-Equiv./Ml.	Cumulative % of Dose Excreted	Mg.-Equiv./Ml.	Cumulative % of Dose Excreted	Mg.-Equiv./Ml.	Cumulative % Dose Excreted
Hours						
0–1	0.1925	8.66	0.4372	1.37	1.3119	22.70
1–3	.4286	30.38	2.2428	14.19	2.5784	61.64
3–6	.4619	61.94	3.0910	33.35	1.2405	82.60
6–9	.3598	76.85	2.3771	50.78	0.4215	89.33
9–12	.2097	84.43	1.6628	64.39	.2454	92.52
12–18	.0518	89.37	0.4396	79.14	.0954	94.83
18–24	.0144	90.27	0.1196	87.56	.0266	95.72
24–30	—	—	.1804	90.33	.0139	96.05
30–36	.0039	90.92	.0740	92.43	.0085	96.29
36–48	.0010	91.03	.0301	93.67	.0018	96.41
48–60	—	—	.0065	94.07	.0014	96.52
60–72	.0005	91.15	.0004	94.24	.0005	96.55
3–4	.0002	91.20	.0001	94.41	Trace	—
4–5	.00010	91.23	.00009	94.50	Trace	—
5–6	.00009	91.25	.00006	94.55	Trace	—
6–7	.00008	91.27	.00002	94.56	Trace	—
10	.00004[a]	—	—	—	—	—
14	.00003[a]	—	—	—	—	—

[a] Standard error greater than ±5%.

Table 5.—Chloroform-extractable metabolites of dimethoate from urine at different pH's.

Time	Total Radioactive Compounds (Mg.-Equiv./Ml.)	At pH 9		At pH 4		At pH 0	
		Mg.-Equiv./ Ml.	Per Cent of Total	Mg.-Equiv./ Ml.	Per Cent of Total	Mg.-Equiv./ Ml.	Per Cent of Total
Hours							
0– 1	0.1925	0.0003	0.16	0.0770	40.00	0.0993	51.58
1– 3	.4286	.0009	.21	.1353	31.57	.1640	38.26
3– 6	.4619	.0006	.13	.1090	23.60	.1311	28.38
6– 9	.3598	.0003	.08	.0426	11.84	.0653	18.15
9–12	.2097	.0003	.14	.0067	3.20	.0086	4.10
12–18	.0518	—	—	.0110	2.12	.0031	5.98

dose was eliminated via this route following oral administration, and about 1.1% when it was given intramuscularly.

Chromatographic analyses of the fecal extracts (6 to 36 hours) from calf 2 demonstrated that 68.8% of the radioactivity behaved as an unknown(s) and 31.2% as dimethoate. Bioassay of fecal extracts demonstrated the presence of compound(s) more toxic to stable flies than the parent compound (Roberts, unpublished data). Almost all the radioactivity in the fecal extracts after the intramuscular treatment ran with the R_f of dimethoate, with only traces of unknown. No mortality was obtained from the fecal extracts of the animal treated intramuscularly.

The results of tissue analyses from calf 1 are given in table 8. The spongy bone and liver contained the highest levels of total radioactive compounds. Assay of the water layer following protein precipitation demonstrated that only the liver and muscle contained significant amounts of water-soluble radioactive compounds. Assay of the chloroform extracts of the tissue homogenates demonstrated significant amounts of the organosoluble radioactive compounds present in the brain, liver, testes, and lungs. Only the brain contained sufficient chloroform-soluble radioactive compounds to allow for further fractionation. Acetone precipitation of the brain extractives demonstrated near quantitative precipitation of the radioactive compound(s) in the phospholipid fraction.

DISCUSSION.—The rapid and near qualitative elimination of dimethoate via the urine and the low levels of radioactive compounds recovered in the feces following oral administration indicate that this compound is very efficiently absorbed from the bovine digestive tract. It appears to be dissimilated by various routes, as evidenced by the large number of metabolites found in the urine with either route of administration. Initially the major metabolite was an unknown which could be extracted from acidified urine with chloroform, but its concentration decreased until by 24 hours it constituted 10 to 16% of the total urine metabolites. Dimethyl phosphoric acid, which was usually found at low concentrations, increased until it became the major metabolite at the end of 18 hours. The concentration of dimethyl phosphorothioate remained fairly constant throughout the first 12 hours. Only trace amounts of dimethyl phosphorodithioate were found, indicating that cleavage of the S—C bond is a minor metabolic pathway. The low concentrations of this metabolite found by chromatography were further substantiated by colorimetric analyses (Norris 1954). Only very low levels of the unchanged dimethoate were found

Table 6.—Per cent of metabolic products in the urine of calves at different times after treatment with P^{32}-dimethoate.

Hours	Dimethyl Phosphate	Dimethyl Phosphoro-thioate	Dimethyl Phosphoro-dithioate	Unknowns				
				1	2	3	4	5
Calf 1								
0– 1	6.7	16.3	1.5	67.3	4.5	3.4		
1– 3	19.9	20.0	1.2	53.3	2.1	3.4		
3– 6	30.4	20.1	2.1	41.7	1.6	4.0		
6– 9	44.6	21.6	1.5	25.7	2.3	4.1		
9–12	64.9	18.2	1.3	9.4	2.5	3.1		
12–18	72.1	7.0	2.9	10.6	2.6	3.1		
Calf 2								
0– 1	1.2	7.2	1.4	87.3	0.32	0.3	0.29	1.9
1– 3	10.7	17.5	2.0	61.5	.45	.6	.13	7.1
3– 6	30.1	22.5	4.0	35.6	.22	1.6	.18	5.0
6– 9	34.0	20.9	3.2	30.8	.40	2.1	.1	8.4
9–12	41.0	24.1	1.3	25.1	.19	2.0	—	6.4
12–18	44.7	23.5	2.2	20.0	.61	4.2	.21	4.5
18–24	46.1	21.3	3.4	15.8	2.6	4.8	—	6.0
24–30	60.6	13.0	5.0	7.9	2.4	4.0	—	7.2
Calf 3								
0– 1	2.0	5.6	2.2	83.6	0.29	0.73	0.20	4.9
1– 3	14.0	22.3	1.5	58.8	.18	1.3	—	2.3
3– 6	36.9	19.5	2.4	32.5	.45	1.5	Trace	6.7
6– 9	61.5	19.1	3.7	9.7	.16	1.1	0.30	4.5
9–12	62.3	20.8	2.4	5.9	1.6	3.2	—	3.9

Table 7.—Radioactive compounds in the feces of calves treated with P^{32}-dimethoate.

Time	Radioactive Compounds (μg.-equiv./gm.)		Cumulative Per Cent of Dose Excreted
	Total	Extractable[a]	
Hours			
Calf 1			
0–3	0.9	0.9	0.09
3–6	4.1	1.8	.45
6–12	10.6	0.9	2.43
12–18	7.2	0.2	3.38
18–24	5.1	0.1	4.24
24–36	2.3	Trace	4.70
36–48	1.0	Trace	4.95
48–60	.2	Trace	4.99
60–72	.1	0	5.01
Calf 2			
0–3	0	0	0
3–6	0.72	0.19	0.08
6–9	3.70	1.36	.20
9–12	5.12	1.34	.24
12–15	6.05	1.18	.48
15–24	7.33	.58	1.20
24–30	10.60	Trace	1.97
30–36	8.70	Trace	2.56
36–48	3.80	—	3.12
48–60	2.89	—	3.50
60–72	2.65	—	3.71
Days			
3–4	Trace	—	—
4–5	0	—	—
Calf 3			
Hours			
0–3	2.20	1.27	0.02
3–6	3.58	2.45	.07
6–9	6.10	.50	.40
9–12	11.08	.29	.70
12–15	8.70	Trace	.94
15–24	1.40	0	1.10
24–30	Trace	—	—

[a] Chloroform-extractable for calf 1 and dichloromethane-extractable for calves 2 and 3.

Table 8.—Radioactive compounds in the tissues of calf 1 two weeks after oral administration of P^{32}-dimethoate at 10 mg./kg.

Tissue	Microgram-Equivalents per Gram of Tissue[a]	
	Total[b]	Chloroform-Extractable[c]
Liver	1.72	0.02
Kidney	0.17	0
Spleen	.15	0
Brain	.31	.07
Spinal cord	.09	0
Testes	.18	.02
Lung	.27	.02
Heart	.09	0
Tongue	.08	0
Gullet	.09	0
Muscle		
Loin	.11	0
Round	.09	0
Shoulder	.10	0
Blood	.03	0
Gall	0	—
Bone:		
Spongy	2.01	—
Compact	0.46	—
Fat:		
Subcutaneous	—	0
Omental	—	0
Perirenal	—	0
Marrow	—	0

[a] Based on wet weight except bone.
[b] As determined from tissue homogenates, significant sensitivity; 0.034 μg.-equiv./gram.
[c] Significant sensitivity for fat 0.007 μg.-equiv./gram, for the remainder of the tissues; 0.015 μg.-equiv./gram.

in the urine by either route of administration.

The conversion of the parent compound *in vivo* to a more toxic compound, as evidenced by a 365-fold increase of anticholinesterase activity and an increased toxicity to stable flies, suggests a possible conversion to its oxygen analog. This hypothesis is further confirmed by its low *in vitro* (IN_{50} for mouse brain ChE, 3.6×10^{-3}) and high *in vivo* anti-ChE activity. When the parent compound was oxidized with hydrogen peroxide (Fukuto *et al.* 1955), a compound was formed which ran with the same R_f value by the chloroform-heptane ethylene glycol system and had about the same IN_{50} against house fly head ChE (9.6×10^{-7}) as the unknown found in the blood.

The rapid rate of elimination and the low concentrations found in the tissues 2 weeks after treatment indicate a low residue hazard for this compound in the bovine body.

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Relative Efficacy of Several Materials for Control of Poultry Ectoparasites[1]

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ABSTRACT

Nine recently developed chemicals were compared as potential control agents for poultry ectoparasites. Field tests, upon which the summary of results is based, were preceded by preliminary laboratory tests of toxicity of the materials to baby chicks and to poultry ectoparasites.

Argas persicus (Oken) was effectively controlled by a 1% emulsion of Sevin® (1-naphthyl-*N*-methyl carbamate) applied as a premise spray in conjunction with a 0.5% emulsion spray applied to the birds at the rate of 1 gallon per 100 birds. Promising but inconclusive results were obtained with Dibrom® (*O,O*-dimethyl-*O*-(1,2-dibromo-2,2-dichloroethyl) phosphate) and Korlan® (*O,O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate) sprays. A 1% Dylox® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate) spray applied to premises and birds gave unsatisfactory control of the fowl tick.

Menacanthus stramineus (Nitz.) was effectively controlled with the following formulations: Dylox solution, 0.5%, spray, 1 gallon per 100 birds; Korlan emulsion, 0.1%, spray, 1 gallon per 100 birds, in conjunction with nest and litter spray; Sevin emulsion, 0.5% spray, 1 gallon per 100 birds; Sevin dust, 0.5%, 4 ounces per 100 birds. Promising but inconclusive results were obtained with 0.25% Korlan suspension spray.

Ornithonyssus sylviarum (C. & F.) was effectively controlled with the following sprays applied at the rate of 1 gallon per 100 birds: Co-Ral® (*O*-(3-chloro-4-methylumbelliferone)*O,O*-diethyl phosphorthioate) suspension, 0.1%; Dibrom emulsion, 0.25%; Korlan emulsion, 0.25%; Sevin emulsion, 0.25%; Sevin suspension, 0.5%. A 5% Sevin dust applied by puff duster at the rate of 4 ounces per 100 birds was also effective. Unsatisfactory control followed use of the following formulations: Tedion® (2,4,4′,5-tetrachlorodiphenyl sulfone, 80% technical) suspension spray, 0.1%, 1 gallon per 100 birds; 3.6 to 4 ounces per 100 birds of 15%. Delphene® (N,N-diethyl-*m*-toluamide) dust, 4% Dibrom dust, or 3% Tedion dust; 0.3 ounces of Santocel (a powdery silica aerogel) dust per 100 birds; 1% suspension of Dowco 109 (*O*-(4-*tert* butyl-2 chlorophenyl) *O*-methyl methyl phosphoramidothioate).

Only a very versatile material can be considered entirely suitable for control of poultry ectoparasites. It must be safely applicable to the birds, it must not impair the production of eggs and meat, it must display good residual activity, and it must combine insecticidal and acaricidal action. Of the pesticides presently used for poultry work, malathion meets these requirements most effectively. Furman & Weinmann (1956) reported on successful experiments with malathion for control of poultry ectoparasites and demonstrated its relative safety. In the same paper they reviewed briefly research by others which eventually led to the acceptance of malathion as a multipurpose poultry insecticide.

Of the materials tested for poultry work since the introduction of malathion, Korlan® (*O,O*-dimethyl *O*-(2,4,5-trichlorophenyl) phosphorothioate) was very promising. Linkfield & Reid (1958) report longer-lasting protection of fowl from ectoparasites following treatment with Korlan than with malathion. Further investigation is needed concerning the safety of using Korlan on poultry.

This report was designed to compare the efficacy of nine chemicals developed since the introduction of malathion to control the following important poultry pests: the fowl tick (*Argas persicus* (Oken)), the chicken body louse (*Menacanthus stramineus* (Nitz)), and the northern fowl mite (*Ornithonyssus sylviarum* (C. & F.)

The tested materials are at present either used for purposes other than poultry ectoparasite control or are not released for commercial use; therefore the field trials reported here were preceded by laboratory screening tests designed to make a preliminary determination of the toxicity of the chemicals to poultry and to the aforementioned parasites.

MATERIALS AND METHODS.—The following materials were selected for study either because their previous usage for pest control indicated a wide spectrum of activity or because previous use on poultry had not clearly shown their actual effectiveness:

Co-Ral® (*O*-(3-chloro-4-methylumbelliferone)*O,O*-diethyl phosphorothioate) (marketed in the United States by the Chemagro Corporation), known during its experimental stage as Bayer 21/199, was available in form of a wettable powder containing 25% of the active ingredient.

Delphene®, (N,N-diethyl-*m*-toluamide) originally developed as an insect repellent by research workers of the U. S. Department of Agriculture, was made available by the manufacturer (Hercules Powder Company) as a 50% emulsion concentrate and as 25% dust.

Dibrom® (California Spray Chemicals Corporation), chemical identity *O,O*-dimethyl-*O*-(1,2-dibromo-2,2-dichloroethyl) phosphate, was available for the present tests as an emulsifiable concentrate containing 8 pounds of technical material per gallon and as a 4% dust.

Dowco 109®, *O*-(4-*tert* butyl-2-chlorophenyl)*O*-methyl methyl phosphoramidothioate) was formulated by the manufacturer (Dow Chemical Compnay) as a wettable powder containing 25% of the active ingredient.

Dylox®, (*O,O*-dimethyl-2,2,2-trichloro-1-hydroxyethyl-phosphonate) (Chemagro Corporation), known as Dipterex or Bayer L 13/59 in the experimental stage, was formulated for the present tests from a soluble powder containing 50% active ingredient.

Korlan (Dow Chemical Company) which contains the active ingredient of Dow-ET-57 was supplied for the present study as a 12% emulsifiable concentrate, a wettable powder containing 25% of the active ingredient, and a 5% dust.

Santocel C, a powdery silica aerogel with insecticidal

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activity due to its abrading and desiccating effect upon the integument of certain arthropods was provided by the Monsanto Chemical Company.

Sevin®, chemical identity 1-naphthyl-*N*-methyl carbamate, was supplied by the Union Carbide Chemicals Company as a 25% emulsifiable concentrate, a wettable powder containing 50% of the active ingredients, and a 5% dust.

Tedion® (2,4,4′,5-tetrachlorodiphenyl sulfone, 80% technical) (Niagara Chemicals Division) was used during the present study as a 3% dust and as a suspension of the 25% wettable powder.

To determine toxicity to poultry the above named materials were applied to baby chicks. Newly hatched birds were kept for a 2-day adaptation period and then treated either with a liquid formulation by submerging the entire body except the head, or with dust by thoroughly rubbing two pinches of the material into the down on back and underside of the bird. Each test group consisted of five birds. For control purposes one group was treated only with the emulsifier component of a customary spray treatment, another group was dipped in plain water, and several groups were left entirely untreated.

In the laboratory the materials were then subjected to preliminary screening tests for efficacy as control agents. Filter papers were treated with known amounts of the insecticides, and the parasites were exposed to these deposits in petri dishes.

In the case of *A. persicus* the test groups, each consisting of five last instar nymphs and adults, were left on the treated surfaces for 3 hours, then placed in clean petri dishes for further observation, thus simulating a field condition where the ticks would have temporary contact with a treated henhouse wall but then return to a hiding place, not reached by the pesticide. The ticks were exposed to both 6-day-old and 14-day-old deposits of various concentrations of the same material to obtain a preliminary picture of residual activity.

M. stramineus was exposed to 6-hour-old deposits of various concentrations of each material in 2 groups, each consisting of 25 adult and last nymphal instar lice. They were collected with an electric suction device from a heavily infested hen and placed immediately on treated filter papers in sealed petri dishes. These were kept in an incubator at 37° C. at slightly under 100% humidity; mortality was recorded hourly. Six hours after initial exposure mortality increased rapidly among all groups, including the 200 untreated controls, because of the removal of the lice from the host. Therefore, a laboratory result was considered "promising" for the purpose of this study only if complete kill of lice was obtained 4 to 5 hours after initial exposure.

In the laboratory, the method described above was also employed to test control of *O. sylviarum* and high natural mortality set in 16 hours after initial exposure. Only those treatments that in the laboratory gave at least 90% kill of mites within 18 hours were tested in the field.

The field tests for control of fowl ticks were made with small farm flocks in pen-type henhouses; body louse control was tested on hens in small flocks both in ground-pen type houses and in elevated, wire floored cages; infestations of northern fowl mites were found and treated during the present study only on large flocks kept in individual, elevated wire cages and all treatments for this parasite were administered through the underside of the cages. Spray applications were made with a 2-gallon compressed air hand sprayer; the nozzle chosen delivered a medium coarse droplet in a spray cone of approximately 60°; all treatments were intended to deliver 1 gallon of liquid per 100 birds. Dusts were applied with a hand puff duster delivering approximately 2 ounces per 100 puffs, varying with the specific weight of the dust; all dust treatments were made at the rate of 2 puffs per bird. Equipment similar to that used in the present experiments is usually found on poultry ranches in central California; apparently poultry men in this area prefer spray treatments and therefore the majority of applications in this study were made with liquid formulations.

RESULTS AND DISCUSSION.—All statements in subsequent paragraphs concerning the efficacy of a material as well as those referring to appropriate concentrations and methods of application are inferred from results of the field tests only. However, those results of the toxicity tests and the laboratory screening experiments which influenced the organization of the field tests are presented here. Several materials which gave little promise in the laboratory were not tested further; they may perform much better under field conditions but this possibility could not be investigated within the scope of this study.

Co-Ral.—Treatment with 0.25% suspension of Co-Ral killed two of five baby chicks; when diluted further to 0.1% no adverse effect was recorded.

In the laboratory Co-Ral gave only partial control of fowl ticks and was comparatively slow in killing chicken body lice; however, among the northern fowl mites exposed to Co-Ral deposits, 100% mortality was recorded within 16 hours.

In the field a 0.1% Co-Ral suspension was applied to three groups of northern fowl mite infested hens and gave satisfactory control (table 1).

Delphene.—In the toxicity tests all chicks died when treated with 20%, 10%, and 5% Delphene emulsion but survived after a strong initial shock when the concentration was lowered to 2%. The 25% dust formulation caused no obvious ill effects on the baby chicks.

In the laboratory Delphene caused no mortality among fowl ticks placed on deposits of 20% emulsion or 25% dust. For control of chicken body lice Delphene did not compare favorably with other less toxic materials. However, northern fowl mites were killed almost instantaneously when placed on deposits of 20% emulsion and 25% dust.

In a field test (table 1) 15% Delphene dust was ineffective in controlling the northern fowl mite.

Dibrom.—Baby chicks were treated with two pinches of 4% Dibrom dust or with 0.1% Dibrom emulsion without showing adverse effects.

Fowl ticks were controlled very effectively in the laboratory with the 4% dust while the 0.15% emulsion gave no control. In the field a 0.5% Dibrom emulsion premise spray produced marked reduction but not elimination of fowl ticks (table 2).

It is felt that *A. persicus* could be controlled effectively with Dibrom if the hens could be confined and a topical application were made along with the premise spray.

Table 1.—Field tests for control of *Ornithonyssus sylviarum*.

Formulation		Concentration (%)	Application Rate	Infestation[a]		Observation Period (Weeks)	Weeks to	
				Pretreatment	Final		Artificial Reinfestation Attempt	Posttreatment Recurrence of Infestation
Co-Ral	S[b]	0.2	1 gal./104	l-m	o	8	6	—
	S	.1	1 gal./98	l	o	8	6	—
	S	.1	1 gal./98	m-l	o	8	6	—
	S	.1	1 gal./96	m	o	2	—	—
Delphene	D	15	3.6 oz./100	m	m	3	—	1
Dibrom	D	4	3.8 oz./100	l-m	m	6	—	1
	D	4	3.7 oz./100	l-m	l-m	6	—	1
	E	0.25	1 gal./120	l-m	o	9	7	—
	E	.1	1 gal./94	m	l	7	5	6
	E	.1	1 gal./94	l-m	l	7	—	4
Korlan	E	0.25	1 gal./116	m	o	13	—	—
	E	.1	1 gal./123	l-m	l	12	—	3
	E	.1	1 gal./90	m-l	l-m	9	—	7
	S	.25	1 gal./120	m	o	6	—	—
	S	.1	1 gal./103	m-h	o	12	—	—
	S	.1	1 gal./96	h-m	l	9	7	8
Santocel	D	100	0.3 oz./100	l-m	l-m	2	—	1
Sevin	D	5	3.8 oz./100	h-m	o	9	—	—
	D	5	4.0 oz./100	m-h	o	9	7	—
	E	0.5	1 gal./94	l	o	9	7	—
	E	.25	1 gal./94	m-h	o	9	—	—
	E	.1	1 gal./90	h	l	7	5	6
	E	.1	1 gal./90	m	l-m	7	—	1
	S	.5	1 gal./88	m	o	9	7	—
	S	.25	1 gal./96	m	o	7	5	—
Tedion	D	3	4.0 oz./100	h-m	m	3	—	1
	S	0.1	1 gal./105	m	l-m	4	—	1

[a] h, Most hens in group heavily infested (blackened feathers around vent, estimated minimum of 2,000 mites per bird).
m, Most hens in group moderately infested (graying feathers around vent, estimated minimum of 500 mites per bird).
l, Most hens in group lightly infested (color of feathers not affected by presence of mites, less than 500 mites counted per bird).
o, All hens in group free of mites.

[b] D, dust; E, emulsion; S, suspension.

The laboratory results with Dibrom for the control of northern fowl mites were promising for the 4% dust as well as for the emulsion in 0.1% and in 0.25% concentration. In field tests good control and long residual effectiveness were obtained with 0.25% spray. Results at lower concentrations were inconsistent to poor (table 1).

Dowco 109.—Baby chicks treated with a 1% suspension of Dowco 109 showed no ill effects. Upon evaluation of the laboratory tests Dowco 109 was not considered promising for field trial; however, it is entirely possible that the laboratory tests conducted in this study could not do justice to the compound's potentialities.

Dylox.—When applied as a 1% aqueous solution Dylox appeared to be harmless to baby chicks.

In the laboratory it gave good control of fowl ticks at the 1% and 0.5% concentration level on 6-day-old deposits but no kill was observed on 14-day-old deposits. This same loss of residual activity was observed in the field tests (table 2) where ticks that apparently had not left their hiding places for 10 or more days after treatment largely survived.

Chicken body lice exposed in the laboratory to deposits of both 1% and 0.5% Dylox spray were killed within 30 minutes. This very promising finding was supported by field test results (table 3).

Northern fowl mites were not controlled by Dylox in the laboratory; therefore no field tests were made.

Korlan.—In toxicity tests neither two pinches of 5% Korlan dust nor Korlan 0.5% suspension produced apparent effects on baby chicks; however, 0.5% emulsion killed three of five birds.

Combined bird and premise sprays of 0.2 to 0.25% Korlan gave promising but inconsistent control of the fowl tick (table 2).

In laboratory tests for control of chicken body lice Korlan gave promising results as a 0.5% and 0.25% suspension as well as in emulsion form at 0.25% and 0.1%.

When testing Korlan in the field for control of body lice the flocks could not be rigidly confined and coverage was uneven. The application resulted in high initial kill followed by rapid reinfestation (table 3). One treatment with Korlan 0.1% emulsion applied to a small infested flock gave complete control up to the end of a 7-week observation period. In this latter case perfect coverage was obtained.

In tests for control of northern fowl mites spray appli-

Table 2.—Field tests for control of *Argas persicus*.

Formulation	Concentration (%)	Treatment	Pretreatment Infestation Count[a]	Observation Period (weeks)	Final Infestation Count[a]
Dibrom emulsion	0.5	House spray[b]	< 1,000	9	52
Dylox solution	1	House and premise spray; poultry spray: 1 gal./100 hens	63	9	30
Korlan emulsion	0.25	House and premise spray; poultry spray: uneven	212	6	65
Korlan suspension	0.2	House and premise spray; poultry spray: 1 gal./106 hens	32	8	0
Sevin emulsion[c]	1	House spray	>1,000	9	0

[a] Numbers represent live ticks found in the same parts of the poultry house during one half hour of searching.
[b] All house and premise treatments were applied to the point of run-off.
[c] Simultaneously with this poultry house treatment the hens were sprayed with 0.5% Sevin emulsion at the rate of 1 gallon per 112 birds and complete control of attached larvae was obtained.

cations of Korlan gave promising results in concentrations of 0.25% and 0.5% (table 1).

Korlan dust was not tested, in order to avoid duplicating extensive work that had just been completed by W. S. Coates, Jr.[3] who generously made his findings available to supplement this study. His data show that Korlan 5% dust applied thoroughly by hand will control northern fowl mites satisfactorily, but when applied by mechanical puff duster (two puffs per bird) the initial kill is not consistently complete.

Santocel C.—Santocel was safely applied to baby chicks; it does not contain an active skin or systemic toxicant and showed no abrasive action on the birds.

The laboratory tests with Santocel gave promising results. All fowl ticks confined with particles of Santocel in petri dishes died gradually over a period of 2 weeks; the dead ticks appeared hard, dry, and brown.

Chicken body lice confined in Santocel-containing petri dishes were dead within 5 hours. Northern fowl mites were not killed within 18 hours when exposed to Santocel in the laboratory.

In the field it was impossible to apply Santocel properly with the available hand duster. The material tends to float in the air, owing to its extreme fineness; it will not penetrate plumage, and light air currents will scatter any deposits of Santocel.

Based on results of our laboratory tests further testing of Santocel for poultry ectoparasite control seems desirable with a more appropriate formulation or method of application.

Sevin.—In toxicity tests a 1% Sevin suspension killed one of five baby chicks. Suspension and emulsion at 0.5% concentration and lower, as well as the 5% dust were used safely.

In the laboratory Sevin performed well in the control of fowl ticks as a 5% dust and as a 0.5% emulsion. A 1% premise spray was successful in the field when accompanied by a 0.5% poultry spray (table 2).

Chicken body lice and northern fowl mites were effectively controlled by Sevin in field tests (tables 3 and 1) as well as in laboratory tests.

Tedion.—Tedion 3% dust and Tedion 0.1% suspension caused no apparent adverse effects when applied to baby chicks.

Neither of the above formulations gave promising results when tested in the laboratory for control of fowl ticks and chicken body lice, but they performed well against the northern fowl mite.

Results obtained with Tedion in the field for control of *O. sylviarum* (table 1) do not confirm those of the laboratory tests. Neither the 3% dust nor the 0.1% suspension gave satisfactory initial kill of the parasites. The 0.1% concentration (suggested by the manufacturer) apparently is not high enough for the control of poultry mites.

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Table 3.—Field tests for control of *Menacanthus stramineus*.

Formulation	Concentration (%)	Treatment	Application Rate	Infestation[a]		Observation Period (Weeks)	Weeks to Posttreatment Recurrence of Infestation
				Pretreatment	Final		
Dylox solution	1	Poultry+house spray[b]	1 gal./82	h	o	8	—
Dylox solution	0.5	Poultry spray	1 gal./85	m	o	8	—
Korlan emulsion	0.25	Poultry+house spray	uneven	m-h	m-h	6	2
Korlan emulsion	0.1	Poultry+premise spray	1 gal./81	m	o	7	—
Korlan suspension	0.2	Poultry+house spray	1 gal./106	l	l	7	6
Sevin dust	5	Hand dusted; 2 puffs/hen	3.9 ounces/100 hens	l-m	o	8	—
Sevin emulsion	0.5	Poultry+house spray	1 gal./112	m-h	o	8	—

[a] h, Most hens in group heavily infested, nits attached to feathers around vent, skin scaly.
m, Most hens in group moderately infested, no nits around vent but lice could be found on all parts of body when combing through plumage with hand.
l, Most hens in group lightly infested, several lice were seen, mainly around vent.
o, All hens in group were free of body lice.
[b] All house and premise treatments were applied to the point of run-off.

In view of the laboratory results tests with higher concentrations seem warranted but could not be carried out within the scope of this study.

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Partial Life Tables for the Black Hills Beetle

FRED B. KNIGHT[1]

ABSTRACT

Life tables have been developed for use in ecological studies of Black Hills beetle (*Dendroctonus ponderosae* Hopk.) populations. These tables reveal very high mortalities among the developing beetles for all classes of infestations. Decreasing infestations suffer more than 99% mortality; static infestations about 97% and increasing infestations more than 90%. The critical mortality period is between April and July of the spring following the initial attack on the trees, late in the life cycle of the insect.

The use of life tables by ecologists in epidemiological studies was intensified after Deevey (1947) made a transition from demographic life tables to ecological life tables. Early life tables were used for human populations. Only within the last decade or two (Morris & Miller 1954) have life tables been treated in textbooks such as that of Allee *et al.* (1949). The limiting factor in the use of life tables for ecological work is the development of suitable techniques for measurement of natural populations. In forest-insect research in North America detailed life tables have been developed for two defoliators (Morris & Miller 1954, Stark 1958).

Allee *et al.* (1949) characterize a life table as follows: "One of the most useful of all numerical aids for the population student is the life table, a device that records in systematic fashion those facts basic to the age distribution of mortality. In short, a life table 'keeps the books on death!' "

LIFE TABLES AND THEIR USES.—The general form of the life tables used here is that of the standard demographic tables (Allee *et al.* 1949). However, the "d_xF" column of Morris and Miller for mortality factors is added and the "e_x" or life expectation column is deleted. The manner of computing "l_x" and "d_x" is similar to that of Morris and Miller; survivorship and natural mortality are measured by periodic sampling of the population. The procedures used in sampling the Black Hills beetle (*Dendroctonus ponderosae* Hopk.) are described in detail by Knight (1959). Six by six-inch bark samples are removed from infested trees between 4 and 7 feet from the ground. Two such samples are removed from each sample tree, one from the north and one from the south surface. The living beetles, dead beetles, parasites and predators in each sample are counted. Also recorded are the entrance holes and the number of inches of egg gallery. Counts from 25 trees resulted in accurate population estimates in the study locations. The life tables presented are not complete because the sampling dates covered periods of several months and the mortality factors are grouped. Additional sampling procedures must be developed before the various mortality factors can be properly separated.

Deevey (1947) illustrates three ways of developing life tables: "(1) Cases where the age of death (d_x) is directly observed for a large and reasonably random sample of the population; (2) cases where the survival (l_x) of a large cohort (born more or less simultaneously) is followed at fairly close intervals throughout its existence; (3) cases where the age structure is obtained from a sample, assumed to be a random sample of the populations, and d_x is inferred from the shrinkage between successive age classes. . . . The first and third types can be used only if one is prepared to assume that the population is stable in time, so that the actual age distribution and the life table age distribution are identical." Because of the nature of the problem the bark beetle life table is a modification of the second method. Periodic survivorship data cannot be obtained on the same individuals at each sampling date because the insects are destroyed while sampling. Survivorship is measured by sampling the same population but not the same individuals. Since the many individuals in a tree are all born within a short period and the only unrestricted stage is the short adult flight period, one sampling procedure is applicable to practically all of the life cycle.

This paper presents average life tables for three types of infestations from several areas; increasing, static, and decreasing. Survivorship curves developed from the averaged data and, for comparative purposes, curves of data from several individual areas are included. The research was confined to infestations in ponderosa pine, so the results cannot be directly applied to lumber and lodgepole pine infestations.

LIFE CYCLE OF INSECT.—A general description of the life cycle of the insect will be of help in following these life tables. The Black Hills beetle has a simple 1-year life cycle (Blackman 1931, Beal 1939). Most of this year is spent in the inner bark of the trees on which the insect feeds. Only green trees or those with fresh, moist inner bark are attacked by the adult beetles. The beetles emerge, fly, and attack chiefly during August. Most of the eggs are laid by mid-September. The eggs require about 10 days to hatch and usually most of the larvae are half grown before winter. Development continues in the spring and the more advanced larvae begin transforming

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to pupae in late May. Some adults are present in early June, but the beetles do not leave the trees until most of the brood has matured in midsummer. The flight usually starts in late July and continues during August.

SAMPLING PERIODS.—The sampling intervals in these partial life tables are broad. However, each sampling period covers a "susceptible"time interval in the cycle rather than a defined life stage or stadium. The sampling periods, in detail, are:

I. The Egg Stage.—This life stage is sampled indirectly by gallery measurement and use of previously recorded egg production data. The number of eggs laid by the adult female per inch of egg gallery is known (DeLeon 1939). DeLeon's studies showed 4.9 eggs per inch in infested ponderosa pine based on intensive work in one locality. It is reasonable to assume that the average number of eggs produced may vary considerably from area to area and from year to year. DeLeon's 4.9 eggs per inch of egg gallery was used as the standard in the present study. The sampling of populations through the year provides opportunity to estimate the inches of egg gallery per square foot for each study area. The number of eggs per square foot was obtained by multiplying the inches of egg gallery per square foot by 4.9. The eggs are laid in September.

II. First Larval Sample.—This sample is taken in mid- to late-October after most of the fall development has occurred. Sampling is considerably simplified at this time because practically all the progeny are in the cambium layer and few, if any, associated insect species are present. The population at this time is considered the overwintering population.

III. Second Larval Sample.—This sampling is done in early April at the time spring development commences. The dead insects cannot be counted but survival is measured and mortality is estimated by subtraction (II minus III). Sampling in III is more difficult than in II because some of the larvae have moved from the cambium layer into the thicker bark.

III-a. Third Larval Sample.—This sample is taken in early June when most of the larvae are in the late instars and some pupae are present. III-a is not included in the average life tables because this sample has been recorded in only two areas of increasing infestation. It is used, however, in the survivorship curves for two of the increasing infestations. Associated insects are numerous.

IV. Adult Stage.—This sampling is done in mid-July before the beetles emerge for flight. At this time practically all the progeny have reached the adult stage and many are ready to emerge. Sampling is relatively simple at this time, although competing species and predators may be numerous.

The four sampling periods are necessary for determining the general fluctuations of Black Hills beetle populations. The comparison of timber losses from year to year gives the trend of the outbreak and a gross estimate of population losses during the flight period. For example, if more beetles emerge from the trees than infested them, one would expect a larger number of trees to be infested during the flight. However, the same number or volume of trees might actually be infested. The difference between expected and actual numbers of infested trees would represent mortality during flight.

Table 1.—Partial life tables for the Black Hills beetle according to infestation trend.

x	l_x	d_xF	d_x	100_{qx}
Sample Period	No. Alive at Start of x (per sq. ft.)	Factors Responsible for d_x	No. Dying during x (per sq. ft.)	d_x as Percentage of l_x
		Decreasing		
I	318	Tree resistance, egg predation, viability, other	150	47
II	168	Weather, competition, other	114	68
III	54	Competition, predators, parasites, other	48	89
IV	6	Weather, predators, tree resistance, other	2 to 6	>33
Generation			314	>99
		Static		
I	397	—	215	54
II	182	—	97	53
III	85	—	60	71
IV	25	—	15	60
Generation			387	97
		Increasing		
I	370	—	175	47
II	195	—	72	37
III	123	—	65	53
IV	58	—	20 to 45	>34
Generation			332	>90

RESULTS.—Partial life tables (table 1) are presented for three classes of infestation: decreasing, static, and increasing. The tables are based on data from 7, 4, and 5 study areas, respectively. The factors responsible for mortality "d_xF" are listed in approximate order of effectiveness. They are listed only for decreasing infestations but the same factors are responsible for mortality in all classes.

Table 1 shows the average population changes for various trends of infestation. It is apparent that the average mortality and the number of insects at the start of period I do not vary in direct accordance with the infestation trend. This fact is also indicated when the number of entrance holes per square foot for the various classes are compared; decreasing, 4.4, static, 4.9, and increasing, 5.2. These figures differ but not significantly. The range between areas is similar for the three classes. The low counts were: decreasing, 3.0, static, 2.2, and increasing, 3.1, and the high counts, 5.3, 6.6, and 7.9. Thus, the number of entrance holes varies considerably from area to area but only the highest counts seem to be related to trend. The overall average number of entrance holes per square foot is approximately five.

The percentage mortality during II and III varies inversely as the trend varies; lowest mortalities occur during the increasing trend. During period IV, the adult stage and flight period, it is more difficult to determine what happens to the insect. Sampling has shown that, on the average, five entrance holes per square foot are made in an infested tree. Thus, in a static infestation about 10 emerging beetles per square foot from the sampling location survive the flight period and make successful egg galleries. In our decreasing table less than 10 are alive at the start of the period. It is estimated that a minimum of 33% and a maximum of 100% would die before successful galleries are formed in a newly infested tree. In the static situation 10 beetles must survive to cause the same amount of tree killing as in the previous year. During increasing trends a minimum of slightly more than 10 for slowly increasing trends and a maximum of about three

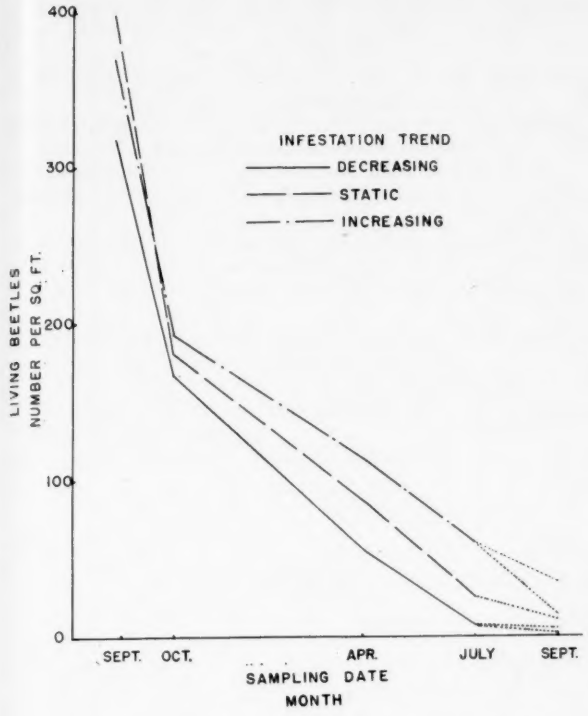

FIG. 1.—Average survivorship curves for the Black Hills beetle by infestation trend.

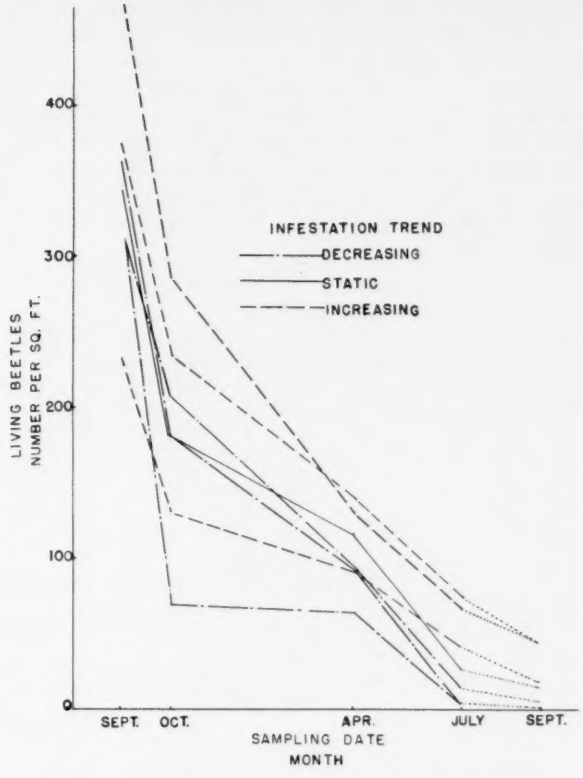

FIG. 2.—Survivorship curves for the Black Hills beetle study areas.

times as much for rapidly increasing infestations would survive. This assumes that a rapidly increasing infestation means about a four to one increase in number of trees infested. In actual situations the increase can be anywhere from slightly more than one to one to more than four to one.

Figures 1, 2, and 3 present survivorship curves, comparing average life data with specific examples. Figure 1 is a presentation of the three average survivorship curves.

Figure 2 contains seven curves showing survival in three of the increasing areas, one static area and three decreasing areas. Figure 3 presents two curves of increasing infestations showing an additional sample (III-a) which is obtained in early June.

The picture is clear considering the average survivorship curves (fig. 1). At the time of fall sampling (October), the populations are in alignment and in April the trend may be indicated. The period of decisive mortality is between October and April, when weather is probably the major factor.

Careful study of figure 2 and a comparison with figure 1 show that the April predictions may not be valid. In April we cannot make accurate trend predictions and the October to April mortality period no longer seems most important. Instead, accurate trend predictions must await the July sample shortly before the beetle flight. The mortality factors which have their effect between April and July determine the final course of the infestation; these factors are primarily biological.

In 1957, it was recognized that trend predictions could not be made accurately in April. Therefore, in 1958 an additional sampling period was added to the study be-

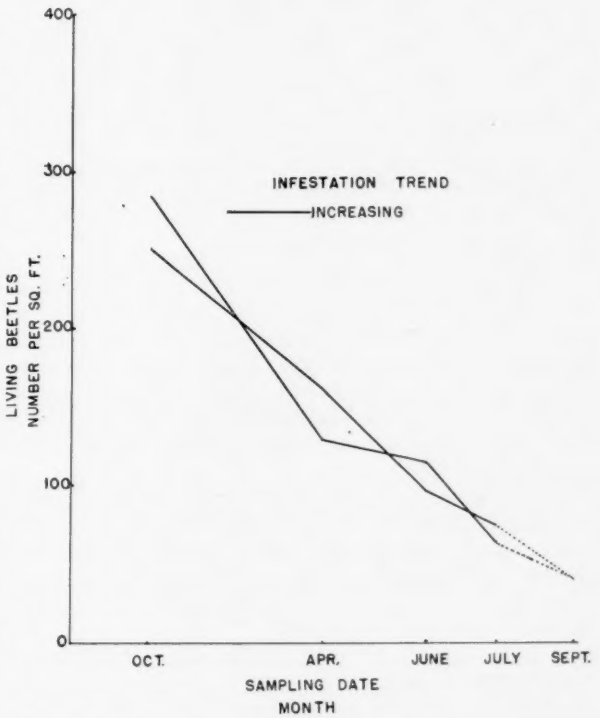

FIG. 3.—Survivorship curves for the Black Hills beetle from two study areas. Infestations increasing—Sample III-a included.

tween April and July. Two study areas were sampled in early June 1958; both were in increasing infestations. The effect of the June sample (III-a) on the survivorship curves is shown in figure 3. The value of this sample for use in trend predictions is not known and evaluation must await the collection of comparable data from static and decreasing infestations. The two examples in figure 3 show quite opposite mortality effects; in one the trend is steep between April and June and in the other between June and July.

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Field Observations on the Effects of Releasing Sterile Screw-worms in Florida[1]

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ABSTRACT

Approximately 500 sterile male screw-worm flies (*Callitroga hominivorax* (Cqrl.)) were released weekly per square mile over a 2,000-square-mile area in Florida. The insects had been irradiated as pupae, within 2 days of adult emergence, with 6,200 to 8,300 r gamma rays from cobalt-60. They were packaged at the rate of 880 (later 550) pupae per release carton. Flies were distributed daily in 6-mile swaths by small aircraft. Shifting of flight lanes resulted in the area being covered in 1-mile swaths weekly. Egg-mass collections in the treated area declined from a weekly average of 41 per station during the first 2 months to 11 in the 12th week. Check stations indicated a continuing high population north of the treated area but a decline on the west and south. However, at the end of 3 months 70% of the egg masses were sterile.

Screw-worms (*Callitroga hominivorax* (Cqrl.)) were not present in the Southeast until 1933, when they apparently were introduced into southern Georgia through the shipment of infested cattle from drought areas in Texas. If the present population were eradicated, the area could be kept free from this insect through inspection and treatment of livestock and pet shipments originating in infested areas.

In 1938 Knipling (1955) suggested the release of sterile males as a method of eradication. Although thorough treatment of livestock with smears would do much to limit the screw-worm population, wildlife would provide a constant reservoir of these insects. Only with sterile males could this reservoir be reached.

Laboratory tests (Bushland & Hopkins 1951, 1953) demonstrated that screw-worms could be sterilized through the use of X-rays or gamma-rays. Mixed-cage populations of sterile and normal flies produced infertile eggs in about the same ratio as sterile to normal males. Although the males from selected progeny have been observed to mate up to 21 times under laboratory conditions, the females mate only once. Subsequent field tests in Florida demonstrated that the sterile males would compete successfully for mates with the native males in nature.

An isolated area with a long record of screw-worm infestation was required to demonstrate eradication. This situation was found on the 170-square-mile island of Curaçao, Netherlands Antilles, 40 miles off the coast of Venezuela. Following preliminary tests the screw-worm was eradicated in 5 months during 1954 through the release of 400 sterile males per square mile per week (Baumhover *et al.* 1955).

Since the treated area in the Southeast during a screw-worm eradication campaign would be about 300 times as large as Curaçao, considerable work was needed to develop large-scale operational procedures. As an intermediate step between the Curaçao test and an eradication program in the Southeast, a 2,000-square-mile test was conducted. The purpose of this paper is to describe (1) the test area, (2) field observations to determine results, (3) sterilization, (4) packaging and holding and (5) release of the flies. Insects were reared by the method described by Graham & Dudley (1959). Various phases of the test were illustrated photographically (Baumhover 1958).

TEST AREA.—Since a screw-worm outbreak occurred over most of Florida, it was possible to select a test area

[1] Accepted for publication June 24, 1959. This work was conducted in cooperation with the Animal Disease Eradication Division, U.S.D.A., and the Florida Livestock Board.

[2] R. C. Bushland, Entomology Research Division, directed the program with the assistance of C. L. Smith. All authors are now employed by the Animal Disease Eradication Division, currently engaged in the Screw-worm Eradication Program in the Southeast.

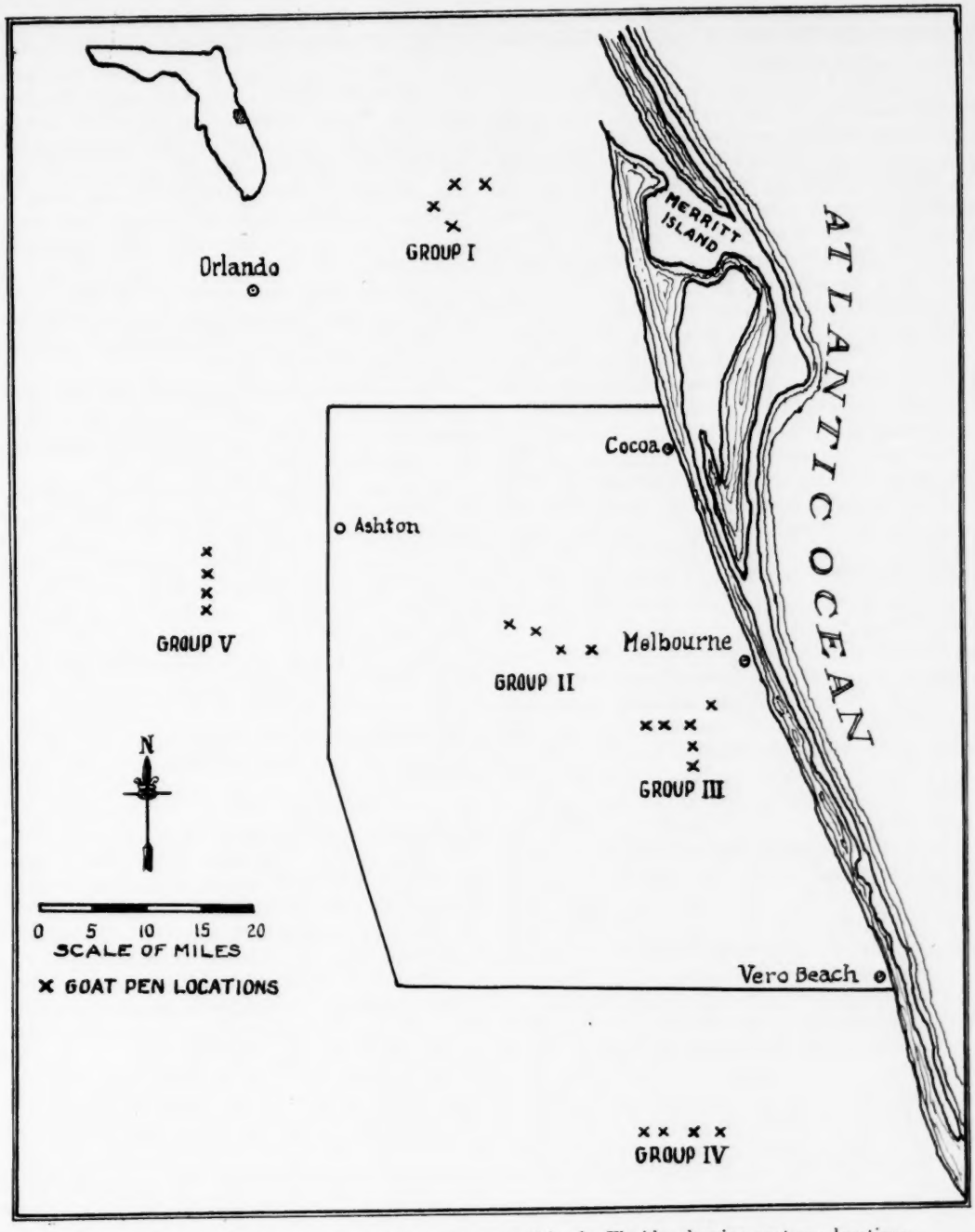

FIG. 1.—Map of sterile-screw-worm release area near Orlando, Florida, showing goat-pen locations.

near the Orlando laboratory (fig. 1). The northern boundary extended westward 30 miles from a point 4 miles north of Cocoa, and the southern boundary 46 miles westward from Vero Beach. On the west the boundary extended 35 miles south from the northwest corner through Ashton to the mideastern shore of Lake Kissimmee, and then followed the Kissimmee River to a point of intersection with the southern boundary paralleling Highway 60. The Atlantic Ocean provided a natural barrier on the east, and it was believed that the prevailing southeasterly winds would reduce migration of flies into the treated area. Although some citrus is grown here, the livestock industry predominates. The test area included 2,000 square miles.

FIELD OBSERVATIONS.—The standard procedure of exposing wounded goats for egg-mass collections was followed to evaluate the activity of both the wild and the released populations. Two groups of goat pens were established in the treated area, and one group 12 to 15 miles from each of the three land boundaries (fig. 1). From four to six goats were maintained in each pen. Groups I, II, IV, and V contained four pens each, and group III con-

tained four pens from May 5 to 25, six from May 26 to July 13, and five from July 14 to August 30. Pens within a group were 2 to 3 miles apart.

Initially two goats in each pen were wounded on the shoulder and infested with approximately 50 screw-worm larvae. An additional goat was wounded every 3 or 4 days to provide constantly three or four attractive wounds in each pen. On the fourth day of larval growth the insects were killed with benzol. The wounds were attractive to ovipositing flies during the infestation and 3 to 4 days after treatment. Often the wounds were reinfested naturally, prolonging the attractive period.

Egg collections were made as late in the day as possible, since screw-worm flies oviposit primarily in daylight. Fertile egg masses laid after the observer visited the pen could be accounted for by the presence of empty shells and newly hatched larvae the next day. Sterile masses retained their original appearance.

To supplement egg-mass records, ranchers in and near the treated area were questioned to obtain case-incidence records. A lack of personnel delayed the survey until the week beginning May 26, so data prior to this date represent estimates. Notebooks were left with the ranchers for recording the number of cases treated each week. Ranchers were interviewed near goat-pen locations and as far as 70 miles from the center of the release area.

STERILIZATION OF FLIES.—For this test a 480-curie cobalt-60 unit was used.[3] Eight strips of cobalt-60 clad in stainless steel, $13\frac{1}{2}\times2\times\frac{1}{8}$ inches finished, uniformly spaced in a cylinder $5\frac{3}{4}$ inches in diameter and shielded by lead, provided the source of radiation. Initially, in April 1957, the average radiation intensity was 1,150 r per minute. The sterilizing dose of 7,500 r therefore required 6.5 minutes' irradiation. Owing to variation within the useful volume, the minimum dose received was 6,200 r and the maximum dose 8,300 r. Correction for decay of the cobalt-60 (half-life 5.3 years) was made each month.

The useful volume of 2 liters allowed the irradiation of about 18,000 insects per load. Irradiation time, including insertions and removal of the canister, was 16 hours for the weekly production goal of 2 million released flies.

As the larvae pupated, they were separated at 12-hour intervals, washed, dried, and held at 80° ± 2° F. and a relative humidity of approximately 95%. The insects were irradiated when the pupae were $5\frac{1}{2}$ to $6\frac{1}{2}$ days old.

PACKAGING AND HOLDING THE FLIES.—Immediately following irradiation the pupae were placed in the release cartons of absorbent paper board, measuring $5\frac{1}{2}\times4\frac{1}{2}\times2$ inches. Partitions placed in the cartons provided 20 cells which were needed to increase resting area for the flies. The fly-resting surface within a carton was about 200 square inches.

For the Curaçao experiment 130 pupae, with an expected yield of 100 flies, had been placed in a No. 1 kraft paper sack. During a Florida field test in 1956 the same number of insects were placed in a frozen-food carton $5\times4\times1\frac{1}{2}$ inches. However, to conserve space and reduce the cost of release containers, consideration was given to increasing the number of flies per unit area. Preliminary tests indicated that the carton used would support 800 flies (emerging from 880 pupae) for 24 hours after emergence began. This packaging rate was used during the first 6 weeks of the field test. However, later tests showed that reduction of this number to 400 to 500 flies resulted in greater longevity and better physical condition, particularly if release was delayed and the flies remained in the carton for more than 24 hours after emergence. The decision to feed the flies before release further limited the carrying capacity owing to the meconium and increased fecal matter present after the flies had fed on the honey provided. For the remainder of the test the 500 loading rate was used. As the pupal mortality was about 10%, the cartons were loaded with 550 pupae. Honey was applied to the screen cover of the carton with a household baster at the rate of 3 to 4 cc. per carton, after the flies had emerged. The flies were kept in the laboratory until emergence was complete and were then released within 24 hours, except when occasional unfavorable weather conditions delayed flights.

Prior to release the packaged flies were held in bun pans 17×25 inches at the rate of 15 cartons per pan. Bun pan racks on casters, holding 15 to 18 pans each, facilitated handling of the cartons.

RELEASE OF FLIES.—The plan of the experiment was to release 500 sterile males per square mile per week, or a weekly total of 2 million flies of both sexes. Owing to a delay in expansion of the rearing facilities, originally designed to rear 1 million screw-worms per week, less than half the 2 million goal was achieved during the period May 1 to 11. During this time the flies were released at the 500 rate only on the eastern portion of the test area. Consequently, only pens in group III were in the release area during this period, group II being on the western boundary of the initial release area. However, from May 12 until the last release of August 17, the weekly rate averaged 548 males per square mile.

Flies were released every day except Sunday. The flight lanes were 6 miles apart, and on succeeding days they were shifted 1 mile to cover the area in mile swaths each week. The airplane flew at an altitude of 1,000 feet except over the coast, at which point the cartons were opened and dropped from 300 feet to assure that the insects reached the ground near the beach.

A modified 125 h.p. Super Cub airplane was used for release. Attached vertically to the back of the front seat was a $2\frac{1}{2}$" by 6" rectangular aluminum release tube which extended through the floor and belly 12 inches below the plane. The release operator, riding in the back seat, held the release carton over the mouth of the tube and freed the lid. Escape of the flies in the plane was prevented by the downdraft through the tube. The carton was immediately dropped into the tube to rest on two "fingers" in a horizontal position near the exit. Flies trickled from the carton until it was released just prior to insertion of the next carton. Rotation of the fingers to a vertical position released the empty carton allowing it to pass through the tube. Release was controlled by the operator with a crank positioned at the top of the tube, and connected to the lower finger assembly with a roller chain and sprocket drive. The metal wall of the baggage compartment in the airplane was replaced with $\frac{1}{2}$-inch hardware cloth to allow movement of air to and through the load of release cartons thus preventing mortality of flies from overheating. Maximum carrying capacity was 300 cartons, and to reduce ferrying time for reloading an air-conditioned sedan

[3] M. E. Jefferson, radiological safety officer, Agricultural Research Service, designed and installed the cobalt-60 source with the assistance of J. F. Mullins of the Soil and Water Conservation Research Division.

Table 1.—Egg-mass records during release of sterilized male screw-worms in east-central Florida, 1957.

Date	Release Area				Check Areas					
	Group II		Group III		Group I (North)		Group IV (South)		Group V (West)	
	Total Number	Per Cent Sterile	Total Number	Per Cent Sterile	Total Number	Per Cent Sterile	Total Number	Per Cent Sterile	Total Number	Per Cent Sterile
May 2–4	75	0	12	0	11	0	—	—	—	—
5–11	214	4.7	39	7.7	61	1.6	61	0	32	0
12–18	283	5.3	11	0	92	4.3	72	0	50	8.0
19–25	151	9.9	47	23.4	146	1.4	100	0.1	44	9.9
26–June 1	119	4.2	188	23.9	184	5.4	94	1.1	43	2.3
June 2–8	182	10.0	376	19.1	140	0.7	102	2.9	45	2.2
9–15	160	8.7	415	25.0	133	1.5	96	2.1	56	0
16–22	186	25.3	333	40.0	200	0	78	0	30	0
23–29	104	25.0	244	42.1	185	1.6	36	5.6	24	4.2
30–July 6	88	27.3	194	40.7	111	3.6	18	0	18	0
July 7–13	70	40.0	103	44.7	121	.8	13	0	6	0
14–20	56	62.5	97	76.3	94	3.2	6	0	8	50.0
21–27	33	69.7	65	69.2	154	11.0	9	0	4	0
28–Aug. 3	5	80.0	15	73.3	133	0	3	0	3	0
Aug. 4–10	11	72.7	14	64.3	112	5.4	10	0	0	—
11–17[a]	12	58.3	38	60.5	125	4.8	20	0	1	0
18–24	0	—	17	52.9	129	5.4	4	50.0	0	—
25–30	4	25.0	14	35.7	99	1.0	14	14.3	0	—

[a] Final release.

delivery automobile transported cartons (in screen-bottom metal trays spaced 1½ inches apart) to strategic landing strips in, or near, the release area.

Laboratory observations were made on samples from each day's flight. One carton was removed from each of the two 12-hour pupation groups released each day, and the pupae were compared with a similar number of pupae that had not been treated. Pupal mortality counts were made after emergence was complete and adult mortality counts after 7 days. Both treated and untreated insects were confined in shell vials containing a small quantity of lean ground beef. Normal screw-worm females held at 95° F. for 2 hours oviposit readily, but females treated at the 7,500 r rate are incapable of oviposition. Absence of eggs verified the fact that only sterile insects were released. Pupal-mortality counts indicated the vigor of the culture as well as forming a basis for estimating the number of flies released. Adult-mortality counts also indicated the vigor of the cultures as well as any adverse effects from irradiation, packaging, holding or release.

RESULTS OF EGG-MASS COLLECTIONS.—Screw-worm activity in the treated area was at outbreak proportions during the first 8 weeks of the test. From May 5 through June 29 the weekly egg-mass collections (table 1) averaged 41 per goat pen in the release area (groups II and III). This was almost 2½ times the weekly average of 17 recorded on Curaçao during the peak of screw-worm activity, which also occurred during the first 8 weeks of that experiment. In group I, 15 miles north of the treated area, the weekly average was 36 masses; in group IV, 14 miles south, 20 masses; and in group V, 12 miles west, 10 masses. According to rancher reports, at least 80% of the calves born during the spring of 1957 suffered navel infestations. Some ranchers said that all their newborn calves became infested with screw-worms.

In spite of continuing high populations in the release area, the sterility increased steadily and during the eighth week of collections highs of 25% in group II and 42.1% in group III were recorded. During the 12th week, beginning July 21, the egg-mass average dropped to eight in group II and 13 in group III. Sterility in both groups approached 70%. Group I remained high with an average of 38 masses, but group IV dropped to an average of 2 and group V to 1 mass per pen.

Data obtained during the last 5 weeks of the test are difficult to interpret. As the total number of egg masses declined, a large per cent of the fertile masses collected inside the release area were undoubtedly from females migrating into the area after having mated with native males. If the release area had been truly isolated, as at Curaçao, almost 100% sterility would have been expected by the time the experiment was terminated.

Egg-mass collections outside the release area indicated considerable fly movement; *e.g.*, during the 12th week 11% of the 154 masses collected from group I were sterile,

Table 2.—Screw-worm incidence records obtained from ranchers in and surrounding sterile screw-worm release area in east-central Florida, 1957.[a]

Date	Release Area	Check Areas			Extended Area[b]	
		North	South	West	Southwest Section	Northwest Section
April	0.6	1.4	1.2	0.5	—	—
May	.6	1.4	1.2	.5	—	—
June 2–8	1.3	2.4	2.6	1.1	—	—
9–15	1.0	1.6	2.2	.6	—	—
16–22	.8	2.9	1.7	.2	—	—
23–29	.7	2.0	0.6	.2	—	—
30–July 6	.7	1.9	.4	.3	—	—
July 7–13	.6	2.7	.3	.1	—	—
14–20	.3	3.0	.2	.1	3.0	—
21–27	.2	3.1	.1	.1	2.1	—
28–Aug. 3	.2	2.8	.2	.1	0.1	—
Aug. 4–10	.05	2.2	.2	.1	.1	4.0
11–17	.05	1.5	.2	.1	.1	3.5
18–24	.05	1.9	.2	.1	.1	3.0
25–31	No record for this period					
Sept. 1–7	.02	1.5	.2	.05	.1	0.3
8–14	.02	0.7	.3	0	.05	.4

[a] Number of screw-worm cases per week per 1,000 acres.
[b] Includes ranches 40 to 70 miles from center of release area.

indicating migration of either the sterile males or wild females after having mated with sterile males, or both. Sterile masses were also collected in the south and west check areas. Field observations indicate that sterility of screw-worm eggs in nature is less than 1%.

RESULTS OF CASE-INCIDENCE SURVEYS.—Weekly case-incidence records (table 2) were obtained from 18 ranchers reporting on 448,560 acres in the release area, 5 ranchers on 22,010 areas in the north check area, 4 ranchers on 74,000 acres in the south check area, and 8 ranchers reporting on 211,880 acres in the west check area. From 13 to 85 ranchers reported weekly on from 59,200 to 652,115 acres in the areas 40 to 70 miles from the center of the release area. These indications of screw-worm activity agreed with the data obtained by collecting egg masses from wounded goats. Screw-worm cases remained high in the north check area, but declined greatly in the release area and in the south and west check areas.

In addition to the valuable experience gained in rearing, sterilizing, and releasing more than 2 million sterile screw-worms per week in a 2,000-square-mile area, these observations provided another demonstration of the effectiveness of such releases. In spite of a severe screw-worm outbreak during the test, 70% sterility was obtained after 3 months of release.

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Residues in Fatty Tissues, Brain, and Milk of Cattle from Insecticides Applied for Grasshopper Control on Rangeland[1]

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ABSTRACT

In 1956 cattle exposed to rangeland treated with aldrin at 2 ounces per acre for different periods averaged 1.4 to 10.9 p.p.m. of dieldrin in the fat at slaughter.

In 1957 steers were exposed for 58 to 90 days to rangeland treated with 2 oz. of aldrin, 3 oz. heptachlor or ¾ oz. of dieldrin per acre then confined in a feed lot for 93 or 100 days. Dieldrin residues from the aldrin treatment were about 4 p.p.m. in the fat when the animals were removed from the range and about 0.9 p.p.m. when slaughtered. Heptachlor epoxide residues from the heptachlor treatment were of the same magnitude. Residues from dieldrin were about 7 p.p.m. when the animals were removed from the range and 1.4 p.p.m. when slaughtered.

In 1958 cattle were grazed for 96 or 103 days on ranges treated with 2 ounces of aldrin or heptachlor per acre in diesel oil or emulsion or 1½ pounds of toxaphene in diesel oil and then confined in a feed lot for 120 days before slaughter. Dieldrin and heptachlor epoxide residues were about the same as found in 1957. Slaughter samples showed that aldrin residues were only slightly affected by the formulation but heptachlor epoxide residues were lowest in animals on the emulsion-treated range. In animals exposed to toxaphene the residues were less than 0.5 p.p.m. Milk from cows exposed to range treated with heptachlor at 2 ounces per acre showed heptachlor epoxide residues of 13.3 p.p.m. in the butterfat (about 0.5 p.p.m. in the milk) after 28 days and 1 p.p.m. after 165 days.

Grasshoppers are important pests of range forage in the western natural-grass States. Insecticides provide the only practical method of control at present for the many species involved. Aldrin, heptachlor, and dieldrin have proved highly effective and economical. The first two are currently recommended, but dieldrin is not recommended because of the persistence of residues on the forage. Toxaphene provided good control but required relatively high rates of application.

All these insecticides will leave residues on forage which may appear in the fat of animals grazed thereon. Aldrin and heptachlor may be changed to their epoxide form. Conversion of heptachlor to its epoxide in the animal body was first reported by Davidow & Randomski (1953). Bann *et al.* (1956) reported the conversion of aldrin to its epoxide, dieldrin, and storage as such in animal fat. The conversion of aldrin and heptachlor to their epoxides in soil was first reported by Gannon & Bigger (1958), and on plants by Gannon & Decker (1958a, 1958b).

In 1954 this Division began a project designed to furnish information on the accumulation of dieldrin in fat of cattle grazing on insecticide-treated rangeland (Hinman

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[2] Because several laboratories and numerous research workers have made important contributions to this work, it is impossible to single out individuals for authorship. The application of insecticides, sampling, and many other tasks were directed by F. T. Cowan, in charge of the Bozeman, Montana, laboratory of the Field Crop Insects and Bee Culture Research Branch. R. V. Connin, F. E. Skoog, J. B. Kreasky, F. V. Lieberman, and W. E. Boundy of the same laboratory assisted in various phases of the project. The chemical analyses were under the general direction of R. H. Carter, of the Pesticide Chemicals Research Branch. Samples of fatty tissue were analyzed by J. E. Fahey and H. V. Rusk at Vincennes, Indiana; soil samples by R. D. Chisholm and L. Koblitsky at Moorestown, New Jersey, samples of range grass and other feeds by K. C. Walker, W. E. Westlake, L. I. Butler, D. A. George, and J. C. Maitlen at Yakima, Washington; and the cream and brain samples by W. E. Westlake, A. J. Ackerman, and R. T. Murphy at Beltsville, Maryland. The Plant Pest Control Division cooperated in this project.

Table 1.—Dieldrin residues in fat of cattle from range treated with aldrin and dieldrin, 1956.

Insecticide	No. Cattle	Date Treated	Date Slaughtered	Dieldrin (p.p.m.) Range	Average
Dieldrin 0.5	7	July 17[a]	Nov. 6	0.3–4.5	2.4
Aldrin 2	8	7	Oct. 8	2.8–7.6	4.5
	7	1	Oct. 8	.9–2.2	1.4
	8	17–22	Oct. 2	2.6–8.1	4.3
	8	17–25	Sept. 10	7.8–14.4	10.9

[a] After Oct. 1 these animals received dry rations in addition to range grass.

1955). This project was interrupted in 1955 by the death of the investigator, but was resumed in 1956 and experiments were continued in 1957 and 1958.

Various insecticides and formulations were applied, and several cattle management practices were followed. Samples of fatty tissue for chemical analysis were obtained each year at slaughter. In 1957 and 1958 biopsy samples were also collected by veterinarians following the method of Radeleff (1950). Samples of range vegetation and soil were also taken in these years. The samples of fatty tissue and vegetation were quick-frozen and shipped by air to the laboratory performing the analyses. In one experiment dairy cows were used to obtain data on residues in milk.

The analytical methods employed for aldrin and dieldrin were those of O'Donnell *et al.* (1954, 1955). Heptachlor was determined by the method of Ordas *et al.*(1956), and heptachlor epoxide and toxaphene by methods developed by the producing companies.

EXPERIMENTS IN 1956.—These investigations were begun after the range treatments had been applied under the Federal-State-rancher cooperative programs. Animals from fenced ranges that had been treated with aldrin or dieldrin were purchased and slaughtered within 3 days after removal from the range, and samples of omental and perineal fatty tissue were collected for residue analysis.

Table 3.—Residues in fatty tissue of steers exposed to range forage treated with different insecticides, 1957.[a]

Insecticide	Oz. per Acre	No. of Animals	Days before Exposure	Days Exposed	Parts per Million of Residue[b] Biopsy	At Slaughter
		Buffalo, Wyoming				
					Dieldrin	
Aldrin	2	8	0	84	4.5	0.6
		4	7	77	3.2	.9
		4	21	63	3.2	.7
Dieldrin	0.75	8	0	83	6.5	1.5
		4	7	76	7.5	1.1
		4	21	62	6.8	1.3
					Heptachlor epoxide	
Heptachlor	3	8	0	83	4.5	1.0
		4	7	76	4.3	.8
		4	21	62	3.3	1.0
		Park County, Montana				
					Dieldrin	
Aldrin	2	8	0	80	5.8	1.8
		4	8	72	3.8	.9
		4	22	58	3.6	.6
Dieldrin	0.75	8	0	90	6.2	1.4
		4	7	83	7.2	1.8
		4	21	69	6.2	1.1
					Heptachlor epoxide	
Heptachlor	3	8	0	87	—	—
		4	7	80	4.4	1.1
		4	21	66	4.1	1.1

[a] In 1 gallon of diesel oil. [b] Corrected for untreated checks.

No aldrin residues were found in the fat of any of the animals. However, as table 1 shows, the average dieldrin residues ranged from 1.4 to 10.9 p.p.m.

EXPERIMENTS IN 1957.—Three fenced pastures in each of two areas, short grass in Wyoming and mixed grass in Montana, were sprayed for grasshopper control with aldrin, heptachlor, or dieldrin in diesel oil. Samples of forage and soil were taken from each pasture at various intervals after treatment. Sixteen steers were grazed on each pasture. Eight were on the pasture at the time of treatment, four were added after 1 week, and four after 3 weeks. Samples of omental fatty tissue were collected by biopsy from all the animals before they were placed on

Table 2.—Residues found in samples of vegetation and soil from rangeland treated with different insecticides, 1957.[a]

Insecticide	Oz. per Acre[b]	Location	Residue Analyzed	Parts per Million After Days Indicated 1	3	7	14	21	42	84
			Vegetation							
Aldrin	2	Wyoming	Aldrin	0.9	0.3	0.1	0.1	0.1	0.1	0.02
			Dieldrin	—	.4	.5	—	—	—	.4
		Montana	Aldrin	.9	.6	.3	.2	.1	.1	—
			Dieldrin	—	.5	.35	—	—	—	.2
Heptachlor	3	Wyoming	Heptachlor	2.1	1.9	.9	1.1	.6	.4	.4
			Heptachlor epoxide	.4	.6	1.0	.9	.5	.3	.2
		Montana	Heptachlor	3.8	1.6	.7	.4	.2	.1	.05
			Heptachlor epoxide	.6	.3	.5	.7	.4	.5	.1
Dieldrin	0.75	Wyoming	Dieldrin	1.8	1.8	1.4	1.5	.8	.4	.3
		Montana	Dieldrin	2.3	1.7	1.4	1.1	1.1	.4	.2
			Soil							
Aldrin	2	Wyoming	Aldrin	1.0	1.0	1.0	1.1	0.9	0.9	1.1
		Montana	Aldrin	.5	—	—	—	—	—	.1
Heptachlor	3	Wyoming	Heptachlor	1.6	1.4	1.2	2.0	1.6	1.4	.9
		Montana	Heptachlor	1.5	—	—	—	—	—	.1
Dieldrin	0.75	Wyoming	Dieldrin	—	.7	.6	.4	.4	.4	.6

[a] Residues on vegetation for aldrin, heptachlor, and heptachlor epoxide analyzed by specific method; those for dieldrin by bioassay; soil analyses made by the organic chlorine method. All results corrected for untreated check.
[b] In 1 gallon of diesel oil.

Table 4.—Residues on range grass treated with different insecticides, 1958.

Insecticide[a]	Residue Analyzed	Pre-treatment (p.p.m.)	Parts per Million After Indicated Days[b]						
			1	3	7	14	21	42	84
Aldrin									
In diesel oil	Aldrin	0	1.1	0.5	0.3	0.2	0.2	0	0
	Dieldrin	0	0.8	1.0	.6	.8	.5	0.7	0.2
In emulsion	Aldrin	0	.3	.2	.1	.1	.2	0	0
	Dieldrin	0	.8	.5	.1	.3	1.0	1.0	0.3
Heptachlor									
In diesel oil	Heptachlor	0.1	1.0	2.2	1.1	.5	.4	.1	0
	Heptachlor epoxide	.1	1.0	1.0	1.2	.5	.4	.8	0.1
In emulsion	Heptachlor	0.1	4.5	0.4	0.2	.1	0	0	0
	Heptachlor epoxide	.1	.4	.4	.5	.1	.2	.3	0
Toxaphene									
In diesel oil	Toxaphene	0	53.0	25.0	24.0	22.0	12.0	11.0	3.0

[a] 2 ounces of toxicant in 1 gallon per acre except for toxaphene, 1½ pounds.
[b] Corrected for untreated check.

treated pasture, and about 90 days after the pastures were treated. The animals were then placed in a feed lot for 93 to 100 days before slaughtering. At slaughter, samples of omental fatty tissue were again collected.

Table 2 summarizes the results of analyses of the vegetation and soil samples. Although the residues on forage samples collected 1 day after treatment were below 4 p.p.m., in some of them 0.4 p.p.m. remained after 84 days. Residues in the soil, which initially ranged from 0.5 to 1.6 p.p.m., decreased only slightly during the study.

The average live weight of the cattle at slaughter time was 897 pounds. Of the 96 animals, 32 graded good, 63 standard, and 1 commercial. The results of analyses of fatty tissue are shown in table 3.

Animals on aldrin-treated range did not contain aldrin residues, but did contain dieldrin. During the exposure period dieldrin residues averaging 4 p.p.m. accumulated in the fatty tissue. They appeared to be somewhat higher in animals exposed from the time of treatment than in those held off the range for the first 1 or 3 weeks. Residues decreased to about 0.9 p.p.m. during the 90 days in the feed lot.

Where dieldrin sprays were applied, about 7 p.p.m. accumulated in fatty tissue during the exposure period. In slaughter samples this residue had decreased to 1.4 p.p.m. In general the residues were as high in the animals held off the range for 7 and 21 days as in those on the range from the date of treatment.

No heptachlor residues were found in the fatty tissue, but during the exposure period about 4 p.p.m. of heptachlor epoxide accumulated. During the 90 days in the feed lot these residues decreased to about 1 p.p.m. There was practically no difference in either the biopsy or slaughter samples between animals exposed immediately and 7 or 21 days after treatment.

Experiments in 1958.—In 1958 aldrin and heptachlor, the two recommended insecticides, and toxaphene were applied for grasshopper control to fenced mixed-grass pastures in the vicinity of Highwood, Montana. The spray treatments and the number of acres in each pasture were as follows:

Aldrin in diesel oil	576
Aldrin emulsion	236
Heptachlor in diesel oil	175
Heptachlor emulsion	186
Toxaphene in diesel oil	113
Untreated	1,700

All applications were made at 1 gallon per acre with light aircraft aided by flaggers and at a flight height of about 15 feet.

Residues in Forage and Soil.—Samples of forage and soil were taken from each pasture before and at various intervals after treatment. Eight sampling sites about equidistant from one another were selected at random in each pasture. At each site forage samples were collected with a reel-type power mower, by cutting the grass from 100-foot strips running at right angles to the direction flown by the spray plane. Three soil samples $4\times6\times\frac{1}{2}$ inches were collected at random from vegetation-free areas at each site, representing about 576 square inches from each pasture.

Table 4 gives the results of analysis of range grass samples. The aldrin residues gradually decreased to zero in 42 days. The dieldrin residues on the aldrin-treated vegetation were variable but persisted throughout the 84-day period. Heptachlor residues from the oil decreased

Table 5.—Residues in soil of rangeland after treatment with different insecticides, 1958.

Insecticide[a]	Residue Analyzed	Parts per Million After Indicated Days[b]					
		1	3	7	14	42	84
Aldrin							
In diesel oil	Aldrin	0.66	0.19	0.15	0.16	0.15	0.22
In emulsion	Aldrin	.64	.25	.23	.24	.26	.24
Heptachlor							
In diesel oil	Heptachlor	.42	.11	.03	.06	0	0
	Heptachlor epoxide	.03	.05	.04	.12	0	0
In emulsion	Heptachlor	1.09	.31	.31	.21	0	0
	Heptachlor epoxide	.05	.11	.13	.19	0	0
Toxaphene							
In diesel oil	Toxaphene	8.59	4.62	3.50	3.61	1.66	.99

[a] Two ounces of toxicant in 1 gallon per acre except for toxaphane, 1½ pounds.
[b] Corrected for untreated check.

Table 6.—Residues found in the fatty tissue of cattle exposed to range forage treated with different insecticides, 1958.

Insecticide	Dosage (Oz./A.)	No. of Animals	Days Exposed	Parts per Million[a] Pre-treatment	Post-treatment	At Slaughter
				Dieldrin		
Aldrin						
In diesel oil	2	5	103	0.1	3.8	1.1[b]
		3	96	0	2.4	0.7
In emulsion	2	5	103	.2	2.8	1.0
		4	96	.1	2.0	0.9
				Heptachlor epoxide		
Heptachlor						
In diesel oil	2	5	103	0	3.7	1.2
		4	96	0	2.7	1.2
In emulsion	2	5	103	0	2.9	.8
		4	96	.1	2.3	.5
				Toxaphene		
Toxaphene						
In diesel oil	24	5	103	.1	3.5	.5
		4	96	.2	3.2	.5

[a] Corrected for untreated checks.
[b] One animal died in feed lot; average of 4 animals.

to zero after 84 days and from the emulsion after 21 days. Heptachlor epoxide residues were present for 42 days when the heptachlor was applied in an emulsion and 84 days in diesel oil. The toxaphene residues decreased rapidly but were present during the 84 days. The feeds used in the dry lot were found to contain none of the insecticides included in this study.

Table 5 summarizes the soil residues. The aldrin residues of about 34% of the dosage 1 day after treatment decreased to 8% after 84 days. The heptachlor residues after 1 day averaged 35% of the amount applied. Both heptachlor and heptachlor epoxide had disappeared from the soil after 42 days. The toxaphene residue was 44% of the dosage after 1 day, and 3% of the dosage after 84 days.

Residues in Fatty Tissue.—Nine cattle were introduced on each pasture, five when sprayed and four 7 days later. Five others were kept on untreated range as checks. All the animals, 21 steers and 29 heifers, were yearlings from the same herd, with no record of previous exposure to insecticides. Pretreatment biopsy samples showed the animals to be free of insecticide contamination. Each treated pasture contained all animals of the same sex, and the untreated range three steers and two heifers. The animals averaged 560 pounds at the start of the experiment and 762 pounds when removed from the range.

As far as possible, each range was checked daily to determine that all animals were on the proper pasture. Colored ear tags greatly facilitated this check. The occasional animal that did go through a fence was immediately returned to its proper place.

Samples of omental fatty tissue were taken by biopsy from all 50 animals before they were placed on the treated pastures, and again 103 days after the range was sprayed. One steer on the pasture treated with aldrin in diesel oil and one on the check pasture died during the exposure period. No post mortems were made on these animals, but the veterinarian who made the biopsies expressed the opinion that both animals had probably been afflicted with pneumonia as calves, and this had contributed to their death rather than any exposure to insecticides. Only eight animals from this pasture were available for biopsy, but a replacement was acquired for the check animal, bringing the total back to five. This replacement was from the same group as the original 50, and had been pastured with the check animals.

Following the biopsies the animals were placed in dry feed lots separated according to sex. A feeding ration of dried beet pulp, barley, mineral supplement, and cured native hay, alfalfa, and wheat straws was supplied. Chemical analyses showed the feed to be free of the insecticides concerned. After 120 days all the animals were slaughtered and samples of omental fatty tissue and brain were collected and analyzed.

One animal from the group exposed on range treated with aldrin in diesel oil died in the feed lot from malfunction of the liver. It is doubtful if exposure to the aldrin contributed in any way to its death.

Residues found in the fatty tissue are given in table 6. The fat in the biopsy samples ranged from 72.8 to 94.1 and in the slaughter samples from 86.1 to 93.3%. A residue of 1 p.p.m. in tissue containing 86.1% of fat would be equal to 1.16 p.p.m. in fat only; in tissue containing 93.3% of fat it would equal 1.07 p.p.m. in fat only.

Of the 48 animals slaughtered, 29 graded choice and 19 good. The average live weights were, 1,086 pounds for the steers and 979 pounds for the heifers. The respective dressed weights were 607 and 533 pounds for yields of 56 and 54%, respectively.

Samples collected from cattle exposed to aldrin-treated pasture were found to contain less than 0.1 p.p.m. of aldrin. Dieldrin residues from the aldrin-in-oil treatment were somewhat higher than those from aldrin emulsion and were also higher in the animals exposed for 103 compared with 96 days. In the samples collected at slaughter there was little, if any, difference due to the exposure period or insecticide formulation.

Biopsy samples collected from cattle exposed to heptachlor-treated range were found to contain less than 0.1 p.p.m. of heptachlor. Animals exposed from the date of treatment averaged 0.6 to 1.0 p.p.m. more residue of heptachlor epoxide than those held off the range for 7 days. The residues in animals foraging on the ranges treated with heptachlor in diesel oil tended to be higher than those exposed to the emulsion. The residues from the samples collected at slaughter were lowest among animals foraging on the emulsion-treated range.

Very little difference existed between biopsy samples from cattle that were not exposed to toxaphene-treated range until 7 days after treatment and those that were exposed immediately. The samples collected at slaughter showed no toxaphene, since the level was less than 0.5 p.p.m. (minimum sensitivity of method).

Residues in Milk.—The rancher on whose land the pastures were located placed four milk cows on the pasture treated with heptachlor in diesel oil. The cows grazed from the date of treatment, and cream samples were obtained before and at various intervals after treatment through the 15th week. All the milk from one milking was put through a separator and 1 pint of the cream taken for analysis. At the end of the 15th week the pasture was enlarged to include an old alfalfa field. Although the animals spent most of their time in the alfalfa stubble, the treated pasture was available to them. Two additional

Table 7.—Heptachlor epoxide residues in milk from cows grazed on range grass sprayed with 2 ounces per acre of heptachlor in diesel oil, 1958.

Days after Treatment	In Butterfat	In Milk[a]
Pre-treatment	0	—
1	2.5	0.1
3	7.9	.3
7	11.6	.4
14	13.6	.5
21	11.8	.4
28	13.3	.5
42	11.2	.4
49	10.2	.4
56	9.3	.4
63	8.0	.3
70	7.8	.3
77	7.1	.3
84	5.9	.2
91	5.5	.2
98	4.7	.2
105	3.4	.1
135	3.4	.1
165	1.0	0.05

[a] Calculated on basis of butterfat content of 4%.

cream samples were collected 30 days apart to determine the reduction in residue when the animals changed to an essentially insecticide-free diet.

The results of the milk sample analyses are given in table 7. The heptachlor epoxide residues reached a peak of about 13 p.p.m. in the butterfat, or about 0.5 p.p.m. in the milk, after 14 to 28 days exposure of the cows on the treated range. Thereafter the residue declined consistently but slowly. After 56 days the residue in the butterfat was above 9 p.p.m. The last sample, collected on the 165th day, showed 1 p.p.m. in the fat, equivalent to less than 0.05 p.p.m. in the milk.

Residues in Brain Tissue.—To find out whether dieldrin and heptachlor epoxide tend to concentrate in brain tissue, samples were obtained from a range cow and her calf that had been exposed on the treated range and from a steer that had not been exposed. Additional samples were obtained from a calf that was not exposed on the treated range but received skim milk from exposed cows containing about 0.02% of butterfat. The cow and her calf had been on the pasture treated with 2 ounces of aldrin in 1 gallon of diesel oil. The cow was on the pasture when it was treated and had a 176-day exposure. Her calf was born 2 weeks after treatment and was therefore exposed for 162 days to the treated range and to milk from the exposed cow. The other calf was kept in a small untreated pasture near the ranch house and was fed for 165 days on skim milk from the four cows that grazed on the heptachlor pasture. The steer was a 2-year-old that had broken a leg as a yearling and had been penned in the feed lot for 13 months.

The animals were slaughtered and samples of omental fat and the entire brain were obtained. The fatty tissue from the cow and calf from the aldrin-treated range contained 4.2 and 3.0 p.p.m. of dieldrin, respectively. The brain tissue contained none. The calf fed on skim milk had 3.1 p.p.m. of heptachlor epoxide in the fatty tissue and 0.3 p.p.m. in the brain. The check steer contained no detectable residues. No reason is known for the large amount of heptachlor epoxide found in the calf fed on skim milk.

Samples of brain tissue were also taken from all the cattle slaughtered after 120 days in the feed lot. Selected samples were analyzed for dieldrin and heptachlor epoxide residues. No dieldrin residues were found in brain tissues from seven animals exposed on aldrin-treated ranges. Samples from four cattle that had grazed on the heptachlor-oil-treated range from the time of treatment until removal 103 days later contained from 0.6 to 1.7 p.p.m. of heptachlor epoxide in the brain tissue. Brain tissue from one animal exposed on the range for 96 days beginning 1 week after treatment contained 0.5 p.p.m. of the epoxide.

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SCIENTIFIC NOTES

Wood Preservatives as Deterrents to Drywood Termites in the Southwest[1]

R. W. HUNT[2]

Wood destruction by subterranean termites and saprophytic fungi has been stressed by building authorities for many decades. Some of the recommended preventive practices are: sanitation, ventilation, insulation, drainage, installation of moisture vapor barriers, use of chemical soil toxicants, and treatment of lumber with preservatives. Why hasn't the home builder been given the same type of guidance in the prevention of drywood termite (*Kalotermes minor* Hagen) attack?

One of the primary reasons is the provincial nature of drywood termite distribution. According to Dr. Thomas E. Snyder's map, published in 1956, the geographical distribution of drywood termites is limited to a narrow band along the south coastal and desert portions of the United States. Although this pest causes serious damage in Florida and in the south half of California, it does not pose a problem of national significance. The cost of control measures in Southern California has now reached proportions comparable to or surpassing that of either subterranean termites or decay.

Drywood termites are not new to Southern California, and, although they range from Mendocino County in the north to San Diego in the south, the area of heaviest infestation is the coastal strip from Santa Barbara south to the Mexican border. (See Kofoid *et al.*, p. 211, 1934.) In the early studies, the prime consideration was damage to utility poles, wooden oil derricks, bridges, and stored lumber. As early as 1914, the writer's attention was called to active drywood termite infestations in the dead limbs of peach trees in an orchard located about 18 miles northeast of San Jose, California. Drywood termites attack soft- and hardwoods alike and seem particularly fond of redwood—especially the spring growth.

Entering the field of structural pest control in 1933, the writer became aware of the extensive damage caused by this pest in the Whittier area, southeast of Los Angeles. At that time, there were extensive walnut plantings in this area, and, as walnut trees are preferred hosts of the drywood termite, many nearby homes were found to be infested.

MATERIALS AND METHODS.—In 1937 the writer built a small office and laboratory, 1 mile east of Whittier, and wishing to protect the structure from the attacks of this insect, sprayed all the Douglas fir framing with a solution of copper sulphate to which a wetting agent had been added. Complete coverage of the framing required approximately 50 gallons of solution applied at a pressure of 275 p. s. i. Now, 22 years after treatment, the visible framing shows no evidence of attack. This is particularly significant because considerable actively infested material has either been brought into the building or has been piled near it.

Largely because of World War II and the Korean War, no further preventive work was undertaken by the writer until 10 years later. Resuming the spray treatments in 1947, the framing of some 30 dwellings and a few industrial buildings have now been treated, with an emulsifiable copper naphthenate being substituted for the copper sulphate used in the original test. Varying amounts and types of chlorinated hydrocarbons were also added and treatments in 1954–55 included the dipping of all cut-ends prior to nailing in place. There were three primary reasons for the change from a solution of copper sulphate to an oil-base emulsive copper naphthenate; first, this formulation was made available by the General Petroleum Corporation; second, it was thought that the oil-base material might give better penetration of wood; and third, it was much easier to handle in the field. The dosage used on all the buildings was 10 gallons of the 20% emulsive copper naphthenate to 90 gallons of water. In seven of these tests, one-half gallon of 25% emulsive DDT was added to each 100-gallon tank, and, in three of the tests 1 quart of 13% emulsive lindane was added to each 100-gallon tank. The total gallonage used on each dwelling varied from 75 gallons for the smaller homes (1000 to 1500 sq. ft.) to 125 gallons for the larger houses (2000 to 2500 sq. ft.).

INSPECTIONS.—Recent inspections of the exposed framing in most of these pretreated structures indicate a strong deterrent action from this relatively thin film of chemical applied to wood members of the structures. Drywood termite wings were observed in the attics of several treated dwellings, but even in these cases, no evidence of attack was visible. The frass of incipient infestation was found in an attic stud of one of the dwellings, but no excrement pellets were in evidence. During construction of this particular building, after treatment, shake siding was applied and fastened with rather long nails. The stud, in question, had split badly where the nails penetrated, exposing untreated wood. It is entirely possible that penetration did not result in the establishing of a colony. The stud has been marked and will be rechecked in the near future. Since this dwelling was sprayed in 1948—some 11 years have elapsed without apparent damage.

The final results of these tests will not be known for some years, but they are very encouraging, especially since coverage was minimal, and the cost of application insignificant.

FIELD OBSERVATIONS.—Field observations of wood structures and utility poles at Navy installations, where both dipped and pressure-treated timber have been used, provide convincing proof of the effective deterrence manifested by wood preservatives against both saprophytic fungi and termites. This was demonstrated particularly in the case of structures built over water. Retention of preservative is one of the primary factors in all types of treatment, and, this factor varies according to species, moisture content, exterior texture and the formulation used. Spraying and brushing result in a very thin deposit, dipping penetrates more deeply, and, pressure-treating proofs the member indefinitely. Regardless of the method used, there is one operation that must be included, that is, the thorough treatment of cut surfaces and drilled holes.

Why is this operation a must? Drywood termites are positively phototrophic during the early hours of swarming, but a complete reversal occurs later, and, after mating they crave darkness. There is generally a small opening where two timbers abutt—such as the juncture of rafter and ridge-board—providing an ideal locale for termite penetration. Spraying can only partially wet the abutting surfaces and even pressure-treated timber has an unprotected center. It is interesting to note some comments by Florida's Dr. E. Morton Miller in 1949. Quote: "Impregnate all wood with an approved rot and termite preservative chemical. Thorough penetration and coverage of all surfaces is necessary. Sometimes such treatment may be combined with a fire-inhibiting chemical. Treatment of bulk lumber which is later sawed and notched by carpenters, thus creating untreated surfaces, is often a waste of money."

DISCUSSION.—One of the obstacles that has delayed the general use of wood preservatives in new construction, is the trite statement that lumber treated with a wood preservative is too expensive. Weighing the likelihood of damage against prevention, we have a definite balance, costwise, in favor of the latter. The costs are directly proportionate to methods and materials used. Spraying and brush-coating with its minimum deposit is least expensive, hot and cold dipping or cold dipping only is more expensive, while pressure-treatment is higher than either of the other methods. Any of these procedures is inex-

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pensive when compared with the costly repairs, treatments and possible fumigation, which may be necessary a few years after a dwelling is built.

AVAILABILITY AND COST.—Most pest control operators are equipped to apply preventive sprays, some individual home owners have set up their own dip-tanks, while others prefer to buy pressure-treated lumber at their neighborhood lumberyards. According to the American Wood Preservers' Institute (1957) pressure-treated lumber was used in the entire framing of a brick-veneer house erected in Jacksonville, Florida, in 1956. The valuation of this residence was $22,000.00, while the extra cost of the treated over untreated lumber was only $462.00 or 2.1%. In calculating the extra cost of pressure-treated lumber to be used in the framing of the house, allow 10 board feet of lumber for every square foot of floor space.

Inquiries at retail lumberyards indicate a variance of from $30 to $47 per 1000 board-feet for pressure-treated lumber as compared with untreated, of the same grade. Since the framing of structures is either partially or completely shielded from the weather, the less expensive water-borne salts are preferred from the standpoint of repellency, paintability, and odor. Some of the salts now in general use are:—chromated zinc chloride (CZC®), fluoride-arsenate-chromate-dinitrophenol (Wolman Salts®), acid copper chromate (Celcure®), chromated zinc arsenate (Boliden®), chromated copper arsenate (Erdalith®), ammonical copper arsenite (Chemonite®), and copperized chromated zinc chloride.

CONCLUSIONS.—Tests and field observations in southern California indicate that the use of relatively small amounts of wood preservative are well worth the cost to the home owner. Any one of the standard water-borne salts was satisfactory for the treatment of the framing of a structure but our tests indicated that a copper component was desirable. Treatment of cut ends, notches, and bored holes is important regardless of the method or material used.

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Thimet as a Low-Temperature Fumigant against the Pea Aphid[1]

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On February 25, 1957, two plots of alfalfa at the Walla Walla, Wash., laboratory, each about 0.2 acre, were treated with granulated 2% Thimet[3] (*O,O*-diethyl *S*-(ethylthio) methyl posphorothioate) at rates of 1 and 2 pounds of active ingredient per acre for control of the pea aphid, *Macrosiphum pisi* (Harris). The granules were broadcast on the soil surface and not worked in. The odor of Thimet was perceptible for at least 10 days after broadcasting. On March 15 four 50-tip samples were taken from each treated plot and eight similar samples from adjacent untreated alfalfa. When examined in the laboratory, the untreated samples averaged 0.40 pea aphid per tip, samples from the 1-pound dosage 0.03, and those from the 2-pound dosage no aphids. At that time it was supposed that the action of the Thimet was systemic, and no further tests were made except to determine a month later that the alfalfa was not toxic to pea aphids. It was suggested, however, that fumigation might be involved, rather than systemic action, because the mortality occurred so soon after treatment, and the dosages used were too low for systemic action. Therefore, on February 21, 1958, twelve 20-×20-foot plots were treated with ½ or 1 pound per acre of 2% Thimet granules. These plots were laid out in randomized blocks, replicated four times. They were made small so that the Thimet vapor might possibly drift from treated to untreated plots. Counts made just before treatment showed a rather evenly distributed population of pea aphids, averaging 0.77 per tip. One week after treatment one 20-tip sample from each plot was examined for aphids. Out of the 12 samples, 1 contained 20 aphids, 2 had 1 aphid each, and the other 9 had no aphids. All samples containing aphids came from untreated plots. The sample containing 20 aphids was from an untreated plot that was up-wind from all the others, and thus escaped fumigation. The aphid mortality on the other untreated plots indicated considerable drift of the Thimet vapor. When these samples were taken, 5 more tips were taken from each plot, stripped down to a single leaf and the terminal bud, caged and infested with 5 aphids each, of various stages. After 48 hours at 77° F. the cages were examined and the aphid mortality and reproduction recorded. Much growth and reproduction had taken place in all samples, and there was no significant difference between the treated and untreated plots. These results would indicate that no detectable absorption or translocation of Thimet had occurred at the time the field mortality was found. The field mortality must have been entirely from fumigation.

Following these tests Thimet was used in a routine manner to kill aphids that had been used to transmit viruses to pea plants. Ten or twelve pots were grouped closely outdoors, and the lids of one or two ice-cream cartons containing granulated Thimet were placed on the edges of the inner pots, without any contact with either plants or soil. The group was then covered with a plastic tarpaulin and left for 48 hours or longer. Complete mortality of the aphids always resulted.

This fumigating action of Thimet against the pea aphid is particularly valuable because it occurs at relatively low temperatures. In the 1957 test the temperature between February 25 and March 15 averaged 44.7° F., with a high of 64° and a low of 29°. In the 1958 test, temperature during the first week averaged 49.1° ranging between 30° and 67°. Most other insecticides tested against pea aphids appear to lose much of their effectiveness at these temperatures, but Thimet apparently retains its killing power under cool conditions.

[1] Accepted for publication March 31, 1959.
[2] The technical assistance of P. E. Featherston is gratefully acknowledged.
[3] Now designated phorate.

A Comparison of Insect Traps Equipped with 6- and 15-Watt Blacklight Lamps[1]

PHILIP W. SMITH, JOHN G. TAYLOR, and JAMES W. APPLE[2]

In recent years entomologists have made increasing use of self-draining, omnidirectional light traps (Taylor *et al.* 1956) equipped with 15-watt blacklight (BL) fluorescent lamps (1950 fluorens).[3] Three years' experience with such a trap in Wisconsin has shown it was satisfactory for collecting many nocturnal species. In 1958 a study was conducted near Arlington, Wisconsin, comparing this trap with a smaller self-draining omnidirectional unit equipped with a 6-watt blacklight lamp (570 fluorens).

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[2] Entomologist, Wisconsin Department of Agriculture, Madison, Agricultural Engineer (deceased), Agricultural Engineering Research Division, U.S.D.A., Purdue University, Lafayette, Indiana; and Professor of Entomology, University of Wisconsin, Madison, respectively. The authors wish to acknowledge the assistance of Mrs. J. H. Esch and Mr. Lloyd Klahn.
[3] Blacklight is a popular name for ultraviolet radiant energy within the range of wave lengths from 3,200 to 4,000 Angstroms. (General Electric Bull. LD-1.)

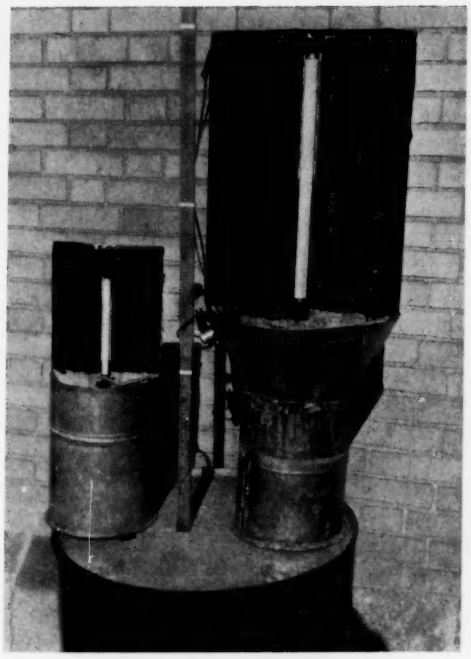

FIG. 1.—6-watt (L) and 15-watt (R) blacklight insect traps used in comparative study at Arlington, Wisconsin, 1958.

This latter trap (fig. 1) was designed and fabricated by the Agricultural Engineering Research Division of the U. S. Department of Agriculture. Each trap had two in-line baffles, rather than four intersecting baffles as suggested by Taylor *et al.*, (loc. cit.). The baffle area of the smaller trap was 30% of that of the 15-watt trap. Following the suggestion of Frost (1957) the baffles were painted black to reduce reflection. The collecting funnel on the 6-watt trap was 8⅛ inches in diameter whereas the larger trap had a 14-inch funnel. For both traps the width of baffles was the same as the diameter of the funnels.

The traps were operated for 34 consecutive nights in the late summer of 1958. Both traps were placed adjacent to corn on the same farm, but about 500 feet apart. Intervening farm buildings minimized the influence one light might have on the other. The traps were placed on the ground and alternated every other night between locations. The two consecutive nights in which a particular trap occupied the two locations are referred to in later discussion as a pair-of-nights. Catches from the traps were emptied each morning, and the more easily identified species were sorted and counted. The total catch of the 19 species involved amounted to 36,365 individuals.

Very poor catches resulting from low temperatures during 13 of the 34 nights made it necessary to eliminate collection data for

Table 1.—Comparison of insect catches in 6-watt and 15-watt BL traps at Arlington, Wisconsin, in 1958.

	No. of Nights	Insects per Trap		Significance of F Values[a]			
		6-W	15-W	Traps	Locations	Paired Nights	Trap X Locations
Agonoderus lecontei Chaud. (seed-corn beetle)	16	1243	2683	N.S.	N.S.	**	*
Agrotis gladaria (Morr.) (clay-backed cutworm)	14	479	906	N.S.	N.S.	**	N.S.
Agrotis venerabilis (Wlk.) (dusky cutworm)	8	60	92	N.S.	N.S.	**	N.S.
Amathes c-nigrum (L.) (spotted cutworm)	16	40	77	*	N.S.	**	N.S.
Caenurgina crassiuscula (Haw.) (clover looper)	16	280	484	*	*	**	N.S.
Crymodes devastator (Brace) (glassy cutworm)	8	167	205	N.S.	N.S.	**	N.S.
Diabrotica longicornis (Say) (northern corn rootworm)	16	1408	2051	N.S.	N.S.	**	N.S.
Feltia ducens (Wlk.) (a noctuid)	16	1327	2320	*	N.S.	**	N.S.
Heliothis zea (Boddie) (corn earworm)	16	35	56	N.S.	N.S.	**	N.S.
Lacinipolia renigera (Steph.) (bristly cutworm)	16	625	849	N.S.	**	*	N.S.
Leucania sp. (a noctuid)	16	74	89	N.S.	N.S.	N.S.	N.S.
Lygus lineolaris (P. de B.) (tarnished plant bug)	16	202	388	N.S.	N.S.	**	*
Nephelodes emmedonia (Cram.) (bronzed cutworm)	14	119	227	*	N.S.	N.S.	N.S.
Melanotrichus flavosporsus (Sahlberg) (lambsquarter plant bug)	16	2099	2039	N.S.	N.S.	**	N.S.
Paraphlepsius irroratus (Say) (irrorate leafhopper)	16	479	989	N.S.	N.S.	**	*
Potamyia flava (Hagan) (a caddis fly)	16	2028	3873	N.S.	N.S.	**	N.S.
Pseudaletia unipuncta (Haw.) (armyworm)	16	80	86	N.S.	N.S.	*	N.S.
Pyrausta nubilalis (Hbn.) (European corn borer)	14	98	156	N.S.	N.S.	**	N.S.
Scotogramma trifolii (Rott.) (clover cutworm)	16	155	225	*	N.S.	**	*

[a] *Significantly different at 5% level. **Significantly different at 1% level. N.S.—nonsignificant.

9 pairs-of-nights. Only the 16 catches were considered where the 10 p.m. temperatures were 60° F. or higher during both nights of a pair-of-nights. Ficht *et al.* (1940) found that at least for the European corn borer, *Pyrausta nubilalis* (Hbn.), temperatures below 60° F. were not conducive for flight. Certain species were absent during part of the effective trapping period, so for these, comparisons were based on less than 8 pairs-of-nights. The nightly catch of each species was converted to logarithms as advocated by Williams (1951) and subjected to an analysis of variance to remove variation associated with trap size, location, pairs-of-nights, and interaction of trap size on location (table 1). The catches used for these analyses included 28,793 individuals, or 79% of the total.

In general the 15-watt trap caught more insects than the smaller 6-watt trap. However, with only five species was this difference great enough to be significant. Only one species was more numerous in the small trap than in the large trap but the difference was of no consequence. The 6-watt trap collected 62.8% as many insects as the 15-watt trap when based on all 14 species showing nonsignificance between traps while the comparable figure for the five species showing a real difference was 56.4%. Significant differences between catches based on pairs-of-nights resulted from a natural diminution of adult activity in late September.

The limited study showed that a small insect trap provided with a 6-watt BL lamp was approximately 60% as efficient as a larger trap equipped with a 15-watt BL lamp. The efficiency of the smaller trap was relatively good considering that the lamp had only 29% of the fluoren output of the 15-watt lamp and that the baffle area of the smaller trap was only 30% of that of the larger trap. However, the diameter of the collecting funnel and the baffle width of the small trap were 58% as large as on the larger trap and these characteristics may have an important relationship to the efficiency of the 6-watt trap. It appeared that in instances where a smaller trap and smaller catches are desired, the 6-watt trap sampled responsive nocturnal insects adequately.

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The Effects of a Diet Containing Gibberellic Acid on the Growth and Food Consumption of *Periplaneta americana L.*[1]

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Gibberellic acid has been employed in modifying the natural growth habit of plants. One can foresee the possible consumption of treated plant material by insects. This study was undertaken to determine the effects of ingested gibberellic acid on the American cockroach (*Periplaneta americana L.*).

Recently Peck *et al.* (1957) published a report on the toxicological evaluation of gibberellic acid. The animal employed in their studies was the laboratory mouse. An intravenous LD_{50} of 6.3 grams per kilogram was obtained and 25 grams per kilogram orally gave minimal signs of toxicity. Other tests such as aerosol application, eye application, and treated diets gave no noticeable effects. Gross and histomorphic studies did not reveal lesions or tissue changes. The authors concluded that Gibberellic acid is relatively harmless when administered orally, topically or even parenterally to the laboratory mouse.

PROCEDURE.—The experimental design employed in this study was a 3×4 factorial with three replicates of each factor. Three classes of 240 insects each based on weight and four levels of gibberellic acid[3] were used.

One class was made up of insects with an average weight of 0.0050 grams, the second class had an average weight of 0.0789 grams and the third class averaged 0.5542 grams. Each weight class represented insects that were uniform in both rearing condition and age (all had hatched within 1 week of other members of the group). Twelve groups of 20 insects within each class were assigned at random to the four treatment levels of gibberellic acid. Quart Mason jars were employed as holding chambers (20 insects per chamber).

The gibberellic acid was incorporated into the cockroach diet chick starter in concentrations of 0.0 p.p.m., 0.01 p.p.m., 1.0 p.p.m. and 100 p.p.m. The appropriate quantity of gibberellic acid was added to 500 ml. of water and then added to 1,000 grams of chick starter. The mash and aqueous solution of gibberellic acid were then thoroughly mixed with a spatula and dried with the aid of an infrared lamp. It was then pulverized with a mortar and pestle. This diet was weighed out for each group of insects and replaced when necessary. The unconsumed portion of the diet was subtracted from the total in calculating total food consumption per insect. The insects were maintained on this diet for 35 days at which time the insects were again counted and weighed. Water was supplied continuously by means of a cotton stoppered test tube suspended in the jar.

RESULTS AND DISCUSSION.—The results obtained have been summarized in tables 1, 2, 3 and 4. The data were subjected to analysis of variance. In each case, the significant differences found at either the 5% or 1% level were those differences between the three weight classes which were different at the begin-

Table 1.—Average weight in grams gained per insect from three different age classes fed four concentrations of gibberellic acid for a 35-day period.

CONCENTRATION (P.P.M.) GIBBERELLIC ACID	WEIGHT CLASSES 1	2	3	MEAN
0	0.0495	0.2765	0.2323	0.1397
0.01	0.0472	0.2677	0.2611	0.1440
1.00	0.0415	0.2747	0.2606	0.1442
100	0.0401	0.3005	0.3100	0.1602
Mean	0.0446	0.2799	0.2685	

Table 2.—Average weight in grams gained per insect for each gram of food consumed from three different age classes fed four concentrations of gibberellic acid for a 35-day period.

CONCENTRATION (P.P.M.) GIBBERELLIC ACID	WEIGHT CLASSES 1	2	3	MEAN
0	0.0431	0.3184	0.6951	0.3732
0.01	0.0460	0.3834	0.8065	0.4119
1.00	0.0373	0.3996	0.7454	0.3941
100	0.0388	0.3960	0.7624	0.3991
Mean	0.0413	0.3901	0.7524	

[1] Accepted for publication July 23, 1959.

[2] Present address: Directorate of Medical Research, Army Chemical Center, Md.

[3] Potassium salt of gibberellic acid, Merck & Co., Rahway, New Jersey.

Table 3.—Average mortality of three replicates (20 insects each) in each weight class fed four concentrations of gibberellic acid for a 35-day period.

CONCENTRATION (P.P.M.) GIBBERELLIC ACID	WEIGHT CLASSES 1	2	3	MEAN
0	2.6667	0.00	1.3333	1.0000
0.01	1.6667	0.6667	0.6667	0.7555
1.00	3.6667	2.0000	1.0000	1.6667
100	2.6667	0.6667	2.3333	1.4002
Mean	2.6667	1.1111	1.8889	

Table 4.—Average number of ootheca produced per female in the oldest age class fed four concentrations of gibberellic acid.

CONCENTRATION (P.P.M.)	OOTHECA/FEMALE
0	1.9107
0.01	1.8758
1.00	1.6508
100	2.1110
Mean	1.8778

ning of the experiment. This relationship was further substantiated by comparing the 0.0 p.p.m. level with the 0.01, 1.0 and 100.0 p.p.m. (levels within the different weight classes). There was no significant interaction between weight and concentration. It is of interest that although the intermediate class consumed the greatest quantity of diet per insect, the heaviest class of insects was most efficient in converting food to body weight. The mortality was quite variable within each weight class and between classes. The highest mortality occurred in the youngest group of roaches.

The data summarized in table 4 are based on female egg production from the heaviest class of cockroaches since this was the only group reaching maturity during the period of the experiment. No significant differences occurred in egg production between the different concentrations of gibberellic acid fed.

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The Economic Importance of *Pleurophorus caesus* Creutz[1]

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It has generally been considered that most species of *Pleurophorus* seldom cause primary injury. Chapin (1940) believed that they are humus-infesting. However, Hatch (1946) reported that E. J. Newcomer found *P. caesus* Creutz infesting tomato and cabbage transplants in a hotbed at Yakima, Wash., in April 1941. Hatch also stated that this little scarab was first recorded in the United States in 1871 and occurs in many Eastern and Midwestern States as well as California and Oregon.

In correspondence dated March 19, 1959, Dr. Hatch listed specimens in the University of Washington collection taken from the following localities: Washington—College Place 1954, Monroe 1939, Moxee City 1939, Seattle 1912, Toppenish 1943, Walla Walla 1941, and Yakima 1941; Oregon—Forest Grove 1938–41, Grants Pass 1947, McMinnville 1938–48, Prineville 1947, and Sucker Creek Canyon (Malheur County) 1951. He stated that another species of *Pleurophorus* was reported by Illingworth (Swezey *et al.* 1931) in the Hawaiian Islands destroying a bed of snapdragons by eating into the stem and girdling the plants just under the ground surface, and that the same species has been recorded about the roots of pineapple plants without mention of injury.

All stages of *P. caesus* were found abundantly in the soil of potato fields in Colorado in 1941 (Henney 1942) and there appeared to be a relationship between the prevalence of this insect and potato scab.

In July 1941 the author found *caesus* in the underground portion of stalks of Russet Burbank potatoes in a field near Wapato, Wash. The infestation appeared to be restricted to plants affected with leaf roll and fungus diseases. Small, white larvae, presumed to have been those of *caesus*, were also found feeding in the pith of the stalks, but efforts to rear them failed. The beetles remained in the dead or dying plants at least until September 5, when the crop was harvested.

P. caesus was rather abundant in many potato fields in the Yakima Valley in 1942, 1943, 1945, and 1948. Adults became active in April and infested seed pieces after planting. Entrance into the seed usually appeared to be through wounds caused by the spearing device of the planters. Diseased potato plants infested with *caesus* were found near Harrah, Wash., on August 27, 1943, and small, white larvae were again present in the stalks but were not reared to maturity.

Adults were found infesting wireworm tunnels in potato tubers on several occasions in the fall of 1942 and were referred to by several growers as the "potato wireworm beetle," probably because it was the only beetle intimately associated with wireworm damage. Potato scab is not a problem in parts of Washington where *caesus* was found. On October 11, 1943, 60 adults, apparently in a hibernating condition, were found crowded together in soil cracks of an abandoned potato field near Toppenish, Wash.; it was therefore inferred that hibernation occurs in infested soil.

On May 14, 1951, adults were seen among the roots of Ladino clover at Toppenish, Wash., but there was no evidence of primary damage. On May 30, 1956, they were found mining the stems of small cucumber plants near Wapato, Wash.; approximately 20% of the plants in a 5-acre field were destroyed.

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[2] In cooperation with the Washington State Potato Commission and the Washington Agricultural Experiment Stations.

A Method for Collecting and Counting Populations of the Shaft Louse[1, 2]

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Several techniques for removing and counting lice from birds have been reported. Beer & Cook (1957) cited a technique for small birds which consists of skinning the bird and placing the

[1] *Menopon gallinae* (L.).

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FIG. 1.—Fumigated chicken from which lice are being fluffed.

skin with attached feathers into a solution of hot KOH and the enzyme trypsin. Everything is digested except the exoskeletons of any lice that are present. The lice are then strained out of the solution with a 200-mesh screen and washed into a petri dish for counting under a stereomicroscope. Floyd & Tower (1956) held a chicken firmly over a large sheet of smooth paper and dusted several pinches of pyrethrum into the feathers. Pyrethrum irritated the lice present causing them to migrate to the surface of the feathers where they are fluffed to the paper by hand. The sample was then stirred into a 100-ml. mixture of water and a wetting agent and poured through a suction filter. The filter paper was placed on a slide mount ringer, covered by a piece of glass marked into five concentric circles and each circle counted separately with a stereomicroscope. Dunn (1932) brushed the feathers after the lice had been anesthetized by the fumes of chloroform applied to a towel draped about the chicken.

None of the techniques were completely satisfactory. Beer & Cook's (1957) method is a lengthy process requiring more than a day for each bird. Also it is difficult to digest all of the feathers and skin of a bird as large as a chicken by this means. In the method cited by Floyd & Tower (1956) the fluffed lice, being alive, tend to wander from the paper. Their counting technique works well only if the sample is voided of all debris, a difficult task. The brushing procedure, (Dunn 1932) is inferior to the fluffing procedure of Floyd & Tower (1956), but lice that are dead or anesthetized are more easily removed than those that are active. Chloroform, in easily handled quantities, will not keep lice anesthetized long enough to complete the brushing.

The method described below was developed during an investigation of populations of lice on chickens at the Virginia Agricultural Experiment Station.

TECHNIQUE FOR COLLECTING LICE.—Each host bird, well secured to prevent any loss of lice by the flopping of wings which accompanies death, was placed in a fumigation chamber to kill the bird and the lice present. A cylindrical cardboard box 8 inches in diameter and 14 inches high with a tight-fitting lid was used as the fumigation chamber; methyl bromide was employed as the fumigant. A 16-mesh galvanized steel wire cloth container, 4½ inches long and 1½ inches in diameter, was filled with cotton, saturated with methyl bromide and placed in the chamber with the chicken. The minimum fumigation time was 3 hours. After removal from the fumigation chamber the chicken was suspended from the ceiling by a wire to the feet, and the wings were locked over the back. A 12-inch by 23-inch polyethylene bag which had previously been threaded up the wire by means of a small hole in the bottom was pulled down over the chicken. The feathers were fluffed with the hand and the polyethylene bag forced the lice to fall straight down to a 4-foot-square piece of smooth paper placed horizontally about 3 inches below the head of the chicken, as shown in figure 1. The lice were then emptied into a sample bottle through a funnel. In preliminary work it was found that more than 85% of the louse population was obtained in the first 10 minutes of fluffing and approximately 95% after the first 15 minutes of fluffing.

TECHNIQUE FOR COUNTING LICE.—All or part of the dry sample was deposited in a petri dish marked with India ink into approximately ⅝-inch squares. Enough xylene was added to wet the sample and a medicinal grade of mineral oil was added until there was a thin layer of the viscous material and lice over the bottom of the petri dish. The lice were then counted a square at a time with the aid of a stereomicroscope using 15 diameters magnification and a mechanical counter. The sample bottle was examined to determine the number of lice missed, if any. The petri dish may be easily cleaned with soft tissue paper, care being taken not to remove the India ink markings.

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Field Tests with Five Insecticides for the Control of Horn Flies[1]

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At the Kerrville, Texas, laboratory, hundreds of chemicals are tested annually to find insecticides that will give better control of biting flies than those currently recommended by the U. S. Department of Agriculture. Although the Department recommends a number of insecticides for control of horn flies (*Siphona irritans* (L.)), their use is limited either by a short period of effectiveness or because of the residues that appear in meat or milk of treated animals.

During the summer of 1958 five insecticides were evaluated under field conditions for the control of horn flies. The test animals were Hereford cattle owned by cooperating ranchers in the vicinity of Kerrville. The various insecticides were sprayed to the point of runoff on each animal and at a pressure of 250 pounds per square inch. Usually the entire herd was sprayed with the

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same insecticide, but where the cattle were pastured in two groups, different insecticides were sometimes used.

The test insecticides were Sevin® (1-naphthyl-*N*-methyl carbamate), Delnav® (2,3-*p*-dioxanedithiol *S,S*-bis (*O,O*-diethyl phosphorodithioate), also known as Hercules AC-528), ronnel (Korlan® grade), Co-Ral (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate, also known as Bayer 21/199), and dicapthon. The effectiveness of these materials was compared with that of the recommended insecticide, methoxychlor.

The horn fly population was estimated before and at various intervals after treatment, and when the average exceeded 25 flies per animal the insecticide was considered as having failed. Test results are presented in table 1.

The summer of 1958 at Kerrville was an excellent one for these tests. Except for about 5 weeks during July and August, the rainfall was conducive to rapid propagation of horn flies. From April to September nearly 22 inches of rain were recorded at the laboratory. The effects of the rain on the test insecticides are difficult to evaluate. In some tests the control periods were longer when the animals were exposed to rain once or twice during the test. It appears, rather, that the reinfestation pressure had a greater effect on the period of protection than did rainfall.

The standard insecticide, methoxychlor, prevented reinfestation for about 21 days. This period is similar to the 2 to 2½ weeks reported by Laake *et al.* (1950) in field tests in Kansas, Missouri, and Oklahoma, and the 4 weeks reported by DeFoliart (1954) in Wyoming. Eddy and Graham (1949) showed that methoxychlor began to lose its effectiveness after the second week. They obtained 100% mortality the first week, 95% the second week, and 54% after 3 weeks when horn flies were released on treated Jerseys confined in screened cages.

Both dicapthon and Sevin failed within the first week, except in one test in which Sevin appeared to protect for 25 days. This test was conducted during that part of the summer when the horn fly population was low.

Delnav prevented reinfestation for periods ranging from 11 days when the reinfestation pressure was high to 49 days when the horn fly population was low, with an average of about 3 weeks. In one test the wettable powder gave better protection than the emulsifiable concentrate, but this was during the drier period of the summer and additional testing will be needed to prove this point. However, Eddy and Graham reported that wettable-powder sprays were more effective than emulsion sprays.

Of the two formulations of ronnel, the wettable powder averaged 30 days and the emulsifiable concentrate averaged 12 days of protection. However, the formulations were not tested concurrently, and it may be that under heavier population pressure the wettable powder will fail sooner than noted in these tests. The 1.0% and 0.5% sprays gave protection periods of about equal duration.

Protection with Co-Ral ranged from 14 to 40 days, with an average of 24 days.

Table 1.—Effectiveness of several insecticides for the control of horn flies.

Insecticide	Per Cent of Active Ingredient	Number of Animals Treated	Number of Flies per Animal Before Treatment	Days' Protection
Sevin, 50% WP	0.5	22	250	7
		11	200	25
		42	100	4
Delnav:				
30% EC	.15	40	75	26[a]
		23	500	11
		44	300	31[a]
		42	300	20
		22	250	15[a]
		54	1200	13[a]
25% WP	.15	40	75	49
Ronnel:				
24.4% EC	.5	10	500	14
		36	1000	11[a]
25% WP	1.0	30	300	30[a]
		16	200	25
	.5	22	100	37[a]
		30	100	28[a]
Co-Ral, 25% WP	.5	31	400	40[a]
		199	75	14[a]
		101	75	21[b]
		27	100	21
Dicapthon, 25% EC	.5	17	500	7
		45	200	6
Methoxychlor, 50% WP (standard)	.5	45	400	18
		27	100	24[a]
		67	400	20[a]
		98	75	21[b]

[a] Rain during test period.
[b] Horn flies were not present at this time, but 2 weeks later the population was high.

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A Comparison of Ronnel and Co-Ral Sprays for Screw-worm Control[1]

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Co-Ral (*O*-(3-chloro-4-methylumbelliferone)*O,O*-diethyl phosphorothioate) was the first insecticide recommended by the U. S. Department of Agriculture for application as a water-base spray for the control of the screw-worm (*Callitroga hominivorax* (Cqrl.)). Previously all the screw-worm control formulations were smears or solutions in volatile solvents intended for application with a paint brush or a pressurized spray applicator.

In both laboratory and field tests with screw-worms, however, it was shown that suspensions of Co-Ral were toxic to all instars at very low concentrations. It was also shown by Kaplanis and associates (unpublished data) in tests with P^{32}-labeled material that the particles would move from one part of a steer's body to another in sufficient quantity to kill the larvae. Field tests indicated that single spray applications were more effective in protecting shear cuts on sheep from screw-worm infestation than were multiple applications of EQ-335 smear (Brundrett & Graham 1958).

In 1958 investigations were undertaken at Kerrville, Texas, to compare several new insecticides with Co-Ral as sprays for screw-worm control. One of the more promising materials was ronnel.

Tests with Artificially Infested Sheep.—In a preliminary test, 34 sheep that had been infested with screw-worms under controlled conditions were sprayed with several concentrations of ronnel. Four sheep were sprayed with 0.25% Co-Ral as a standard of comparison. At the time of treatment all the sheep had one wound infested with 50 to 250 four-day-old larvae in the

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Table 1.—Prevention of natural screw-worm infestation in dehorned steers with sprays, in southwestern Texas, 1958.

Treatment	Number of Screw-Worm Cases	Days Before Infestation	
		Range	Average
Encinal Test (8 steers given each treatment)			
Co-Ral 0.25%	4	8–25	14
0.5%	2	21–25	23
0.5%[a]	4	12–33	23
Ronnel 0.5%	3	11	11
1.0%	4	13–28	21
1.0%[a]	3	15–28	19
Batesville Test (61 steers given each spray, 71 given smear)			
Co-Ral 0.5%	4	8–18	13
Ronnel 1.0%	10	8–12	10
Smear EQ-335[b]	14	3–6	4
Uvalde Test (65 steers given each treatment)			
Co-Ral 0.5%	5	9–12	10
Ronnel 0.5%	8	9–16	11

[a] Head and neck only.
[b] 4 applications.

muscle of the hind leg. A power sprayer was used to apply 1 gallon per animal. The entire body was thoroughly wetted, the wound at the same rate as the other parts of body. When the sheep were examined 24 hours after treatment, all the larvae were dead.

In order to estimate the residual effectiveness of the insecticides, each wound was exposed to reinfestation three times each week until it was reinfested with young larvae or until it healed. Reinfestation was attempted by exposing the sheep to gravid female flies in a screened enclosure until each wound had at least one average-sized egg mass (estimated 200 to 300 eggs). Moist wounds were very attractive to the flies, but after the wounds had healed sufficiently to be dry, it was not always possible to induce oviposition.

All four sheep treated with Co-Ral healed without reinfestation. Ronnel also gave good protection, as 2 sheep sprayed with 2.5% and 11 of the 13 sheep treated with 1% healed without reinfestation. The other two sheep were infested on the 8th and 14th days, a longer period of protection than is obtained by using EQ-335 smear under similar conditions. Four of the 12 treated with 0.25% and 2 of 9 treated with 0.5% ronnel became reinfested.

The number of days required for each wound to heal was recorded, but healing was influenced by several factors other than the insecticide treatment, and the number of animals in each group was inadequate to overcome this variation.

RANCH TESTS.—The results with artificially infested sheep indicated that trials with larger numbers of animals should be undertaken under field conditions. In April 1958 tests were started at Encinal, Texas, with freshly dehorned yearling cattle. Six groups of eight yearlings were used. Two concentrations of each insecticide were tested. At the higher concentration an additional group was sprayed on the head and neck only. In all groups at least half of the cattle healed without becoming infested and the infested animals were protected for about 18 days (table 1). The data indicated that 1% ronnel and 0.5% Co-Ral were about equal as protective applications.

The Encinal test was conducted during a period of heavier than average rainfall, and screw-worm adults were active through out the test period. Comparable cattle in the same pasture were treated with Smear No. 62 and EQ-335. Six of eight treated with Smear No. 62 were infested in about 8 days. The eight yearlings treated with EQ-335 developed 10 infestations in 10 days.

In another test with dehorned cattle at Batesville, Texas, 193 steers were sprayed on June 13, 1958. All the steers had been fire branded 2 weeks before, and the brands were beginning to peel at the time of spraying. Almost 90% of the animals were dehorned and about 60% castrated 1 day before treatment.

The cattle were divided at random into three groups and treated with 0.5% Co-Ral, 1.0% ronnel spray, and EQ-335 smear. A power sprayer operating at 250 pounds per square inch was used to apply 1 to 1¼ gallons to each steer. All the animals were thoroughly wetted, with a special effort being made to wet the scrotum and adjacent areas of the castrated animals. All the cattle were examined carefully in a pen four times during the 18-day test period, and, following the rancher's standard practice, the cattle in the EQ-335 group were re-treated each time the cattle were penned. The sprayed cattle were not re-treated except that the infested animals were treated with EQ-335 and removed from the test. The treatments were subjected to an unusual amount of rain, 0.5 inch on June 15, 5.5 inches on June 17–18, and 2.1 inches on June 23. Large numbers of untreated cattle in adjacent pastures were being infested every day, indicating moderate to severe screw-worm activity.

As shown in table 1, Co-Ral gave better protection than ronnel, but both spray treatments, applied once, gave better protection than four applications of EQ-335.

In a similar test conducted at Uvalde, Texas, in November 1958, 130 newly dehorned steers were divided into two groups, one of which was sprayed with 0.5% Co-Ral and the other with 0.5% ronnel. These animals were inspected twice each week until the test was discontinued on the 27th day because cool weather was inhibiting fly activity. Data in table 1 show that although slightly better results were obtained with Co-Ral, both materials gave about equal protection against screw-worm infestation.

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Control of the Leaf Miner *Profenusa canadensis* (Marlatt) on Cockspur Hawthorns[1]

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Profenusa canadensis (Marlatt), often referred to as the cherry, hawthorn, or cherry and hawthorn sawfly leaf miner, is a serious pest of the English Morello cherry, *Prunus cerasus* var. *austeria* L., and of the cockspur hawthorn, *Cratraegus crus-galli* L. The destructive stage of this pest is a greenish larva that eats the tissue between the epidermal layers of a leaf. In the case of serious infestations of *Profenusa canadensis*, one, two, or even three sawfly leaf miners may be present in a high percentage of the leaves and a substantial part of the foliage present during the early part of the growing season may be destroyed.

Parrott & Fulton (1915) presented a detailed account of the life history, behavior, and morphology of this pest in New York state. At that time they recommended nicotine and soap applied when the young larvae began tunneling in the leaf tissue. Martin (1939) also reported fairly satisfactory control of this leaf miner on English Morello cherries with sprays containing nicotine and soap. Hamilton (1943) obtained a fair measure of control with a spray mixture containing an aliphatic thiocyanate, summer oil, cube root, and blood albumen, applied when the larval tunnels were about one quarter inch long. Later, Hamilton (1950) obtained excellent control of *P. canadensis* on English Morello cherries with BHC, lindane, and chlordane sprays. Hamilton concluded that the optimum time to spray was when most of the

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eggs had hatched. Tomlinson & Rusden (1952–53) reported a reduction in the number of mines in the leaves of hawthorn trees that were treated with lindane, aldrin, and dieldrin sprays applied at the time a few larvae had hatched. However, they did not obtain satisfactory control of larvae of *P. canadensis* in the leaves with these sprays. These authors concluded that, with better timing, the materials which they tested might have proved more effective.

LIFE HISTORY AND HABITS.—*P. canadensis* overwinters as a larva in an earthen cell about 2 inches in the soil beneath infested trees. Pupation occurs in the early spring. The adults are black with an orange-colored spot on the prothorax and are about one-eighth inch long. In Urbana the adults emerge in late April. If the weather is bright they emerge, mate, and lay eggs within about a week. The eggs are somewhat flattened and elliptical and are usually laid near the edges of the leaves on the undersides. In a few days the eggs hatch and the small larvae enter the leaves and form serpentine mines. The larvae grow very rapidly and soon each larva destroys about one-third of the tissue between the surfaces of a leaf. The injury appears as a dead brown blister. By about May 20, most of the larvae have abandoned their mines and dropped to the ground where they form their earthen cells. There is one generation of *P. canadensis* per year and all of its above-ground activity occurs within a month or less.

HOSTS.—The English Morello cherry is a commonly planted variety of sour cherry. The cockspur hawthorn is a small tree or shrub with sharply serrate, ovate leaves and with many brownish spines about 1½ inches in length. Of the nine major groups of *Crataegus* commonly planted for ornamental purposes in Illinois, it appears that only the cockspur hawthorn or *C. crus-galli* group is damaged to any extent by *P. canadensis*. Because this sawfly is so discriminating in its choice of hosts, a very practical control measure for this pest would be to avoid planting the English Morello cherry or the hawthorns of the *C. crus-galli* group.

EXPERIMENTS WITH SPRAYS.—Hawthorns which had been heavily damaged by *P. canadensis* for several years on the campus of the University of Illinois, were made available for the tests. These trees were 8 to 10 feet high and had a spread of 12 to 14 feet.

The first experiment, conducted in 1956, was designed mainly to determine when the spray should be applied. The sprays were applied with a power sprayer developing 400 p.s.i., and equipped with a manually operated spray gun. One application of each of three materials was made on a separate tree each week for 4 weeks beginning April 23 when the leaf buds were beginning to unfold.

When the mining activities were about complete, records were taken by counting the number of mined leaves within the area of an 18-inch hoop held against the tree at eye level on each of four sides. From the data obtained (table 1) it was evident that malathion did not show much promise, but lindane and dieldrin seemed promising when applied April 30 and May 7. April 23 appeared to be a little too early for effective spraying, while May 14, after the mines were formed, was too late. On April 30, the leaves were fully expanded and some new shoots were 5 to 6 inches long.

Table 1.—Results with sprays applied at various dates for control of *Profenusa canadensis*.

INSECTICIDE (ONE QUART PER 100 GAL. OF WATER	NO.[a] OF MINED LEAVES ON TREES SPRAYED			
	April 23	April 30	May 7	May 14
Untreated	3	155	25	27
Malathion[b]	16	88	49	42
Lindane[c]	10	7	1	76
Dieldrin[d]	5	0	0	204

[a] Within four 18-inch circles.
[b] 57% emulsifiable concentrate.
[c] 20% emulsifiable concentrate.
[d] 15% emulsifiable concentrate.

Table 2.—Results with sprays applied three seasons for control of *Profenusa canadensis*.

INSECTICIDE (ONE QUART PER 100 GAL OF WATER	NO.[a] OF MINED LEAVES ON TREES IN			THREE-YEAR AVERAGE
	1957	1958	1959	
Untreated	356	239	208	267.7
Lindane[b]	26	22	16	21.3
Dieldrin[c]	1	6	0	2.3
Aldrin[d]	28	25	14	22.3
L.S.D. 1% level[e]				42.4

[a] Within four 18-inch circles.
[b] 20% emulsifiable concentrate.
[c] 15% emulsifiable concentrate.
[d] 23.5% emulsifiable concentrate.
[e] The authors are indebted to John D. Paschke for statistical analysis of the data.

Beginning in 1957, the plots used in 1956 were re-designed to provide one tree for a control and for each of three sprays in six blocks. Lindane, dieldrin, and aldrin, were applied with a power sprayer as in 1956 and the records were taken in the same manner. All three sprays gave acceptable results each year as shown by the results presented in table 2. Dieldrin was consistently better than lindane and aldrin, giving excellent results.

DISCUSSION.—This study and the studies of the earlier workers show that timing is the most critical factor in the control of *P. canadensis*. Present for only a few weeks, this pest can cause severe damage to hawthorn foliage. When applied at the proper time, dieldrin gave the best control; however, lindane and aldrin were also effective. Treatments should be applied when the leaves are nearly grown and the new shoots are 4 to 6 inches long. This stage is usually reached in the last few days of April in central Illinois.

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Evaluation of Insecticides for Tomato Suckfly Control[1]

JAMES A. HARDING[2]

Suckflies, are mainly pests of tobacco as noted by Brown (1950) and Gilmore (1949, 1950), but are often detrimental to tomatoes as noted by Jones (1933) and Clark (1934), who obtained control with dusting sulfur. Gilmore's work (1949, 1950) with the suckfly on tobacco showed that one quarter pound of

[1] Technical Contribution No. 3292. Texas Agriculturai Experiment Station. Accepted for publication July 31, 1959.
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parathion, 1 pint of 10% tetraethyl pyrophosphate solution in 100 gallons of water at the rate of 80 gallons per acre, and 12 to 15 pounds of a 20% toxaphene dust were effective, but only for a few days. Wene (1949) found that dusts containing DDT-sulphur chlordane, methoxychlor, lindane, toxaphene or parathion were effective in controlling suckflies on tomatoes in the Lower Rio Grande Valley of Texas. The object of the present study was to compare 16 of the more recently developed insecticides for control of the tomato suckfly, *Crytopeltis notatus* (Distant), attacking tomatoes.

Suckflies increased on tomatoes in the Winter Garden area of Texas during late April, 1959. By early May the insects ranged from 7 to 29 individuals per plant with their damage being apparent. Severe chlorosis of the leaves, black excrement, and dwarfing of heavily infested plants were evident. Feeding was mostly on the undersides of the leaves but about 35% of the insects could be found on the upper surfaces at any time of the day. Copulation seemed to take place only in the early morning or when it was relatively cool.

MATERIALS AND METHODS.—Homestead 24 tomato plants were used in the two tests of May 6 and 8. The plants ranged from 10 to 27 inches high averaging 22 inches. Furrow irrigation was used with rows located on beds having 36-inch centers. The mean maximum temperature was 90.6° F., the mean minimum temperature was 65.6° F., the mean temperature was 78.1° F., and the mean relative humidity was 62.6% during this period. No heavy dew or rainfall was recorded during these tests.

The design of each test was a randomized block having each treatment replicated four times. Each plot was 2 rows wide and 60 to 70 feet long. The sampling procedure consisted of striking each of 10 randomly-selected plants per plot 3 to 5 times with a clipboard onto a 16½-by 12-by ½-inch white, metal pan beneath the plant. The insect would be knocked from the plant and stand in the pan for a short period which made counting of the individuals relatively simple. The number of insects was then recorded. Counts were made at 1-, 3-, and 7-day intervals following application. The mean number of suckflies per sample in the untreated plots is shown in the table, column designated as "check." The data were analyzed statistically for sources of variance by applying the "F" test and differences between treatment means were analyzed by the use of Duncan's New-Multiple-Range-Test.

Table 1.—Chemical control of tomato suckflies on tomatoes in 1959.

Treatment		Per Cent Control[a] (Days After Application)		
Material	Pounds Active per Acre	1	3	7
	Experiment 1			
Parathion	0.5	99.5	95.3	0.0
Malathion	1	99.5	97.6	20.5
Diazinon[c]	0.5	98.2	93.9	5.0
Phosdrin	0.5	98.2	98.1	13.8
Guthion	0.5	98.1	90.0	45.3
Trithion	0.75	92.6	91.2	19.0
Methyl-Trithion	0.75	90.1	96.5	36.1
Ethion	0.5	99.5	92.1	22.3
Check	0	9.1[b]	9.5[b]	9.1[b]
	Experiment 2			
Perthane	1	15.1	57.1	50.9
Ronnel	0.75	89.3	77.6	53.6
Thiodan	0.75	87.7	77.6	54.9
Dylox	0.5	81.4	77.1	44.2
Dibrom	0.5	47.2	17.4	9.1
Demeton	0.25	79.2	77.8	64.2
Methoxychlor	0.75	20.4	21.6	—
Dimethoate	0.5	88.1	89.7	75.5
Check	0	8.0[b]	10.1[b]	15.4[b]

[a] Based on untreated check plots.
[b] Mean number of suckflies per plant sample.
[c] Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate).

The emulsion formulations were applied at the rates indicated in the table with a spray volume of 5 gallons per acre at a pressure of 30 p.s.i. by one nozzle per row directly over the plants.

RESULTS AND DISCUSSION.—Results of the first experiment are summarized in table 1. Statistical analysis indicated that 1 day after treatment Trithion®, (*S*(*p*-chlorophenylthio)methyl *O,O*,-diethyl phosphorodithioate), and methyl-Trithion®, (*O-O*-dimethyl(*p*-chlorophenylthio)methyl phosphorodithioate) were less effective than the other chemicals. All treatments were highly effective 3 days after application, but no differences appeared among the insecticides. At 7 days there were no significant differences between treatments but Guthion® (*O,O*-dimethyl *S*-(4-oxo-3H-1,2,3-benzotriazine-3-methyl)phosphorodithioate) and methyl-Trithion seemed to have the longest and most effective residual qualities.

Analysis of the data in the second experiment (table 1) indicated that Perthane® (1,1-dichloro-2,2-bis(*p*-ethylphenyl)ethane and methoxychlor were ineffective; ronnel, Thiodan® (6,7,8,9,10, 10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6, 9-methano-2,4,3-benzodioxathiepin-3-oxide), Dylox® (*O,O*,dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate), and demeton were more effective than Perthane, Dibrom® (*O,O*,-dimethyl-0-(1,2-dibromo-2,2-dichloroethyl)phosphate), and methoyxchlor. Seven-day counts indicated dimethoate was again superior to the other chemicals with demeton second in effectiveness; then Perthane, ronnel, and Thiodan were next in effectiveness. Dylox was inferior to these but was superior to Dibrom or methoxychlor.

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Tests with Insecticides for Control of the Winter Tick[1]

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The winter tick (*Dermacentor albipictus* (Pack.)), often a serious pest of cattle, horses, and other livestock, is found in Canada, most Northern and several Western States. In the Southwest it infests livestock principally in the winter months. Around Kerrville, Texas, infestation may be found from late November through February.

Since all three parasitic stages of the tick are found on livestock, treatments should have residual effectiveness to kill adults and nymphs emerging from nymphal and larval exuviae. One application of an insecticide recommended by the Entomology Research Division (1959) will kill all stages present at the time of spraying and may afford protection throughout the tick season. If the treatment is applied early in the season, a second application may be necessary.

Tests were conducted on several ranches in the vicinity of Kerrville during the winters of 1957–58 and 1958–59 to deter-

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mine initial kill and residual effectiveness afforded by certain new organic phosphorus and carbamate insecticides.

Herds were composed of Hereford cattle and maintained on pasture according to local range practices. The most heavily infested animals of each herd were placed in groups of five, and each group was identified with a marking dye. At each ranch two or more groups were sprayed with test insecticides and one with 0.5% toxaphene, the standard treatment. The animals not included in the tests were also sprayed with 0.5% toxaphene.

The cattle were examined individually, and the numbers of adult ticks on an area of the dewlap and on the entire escutcheon were recorded before and after treatment.

Insecticides were applied with power sprayers operating at a pressure of 100 pounds per square inch in 1957 and 250 pounds in 1958. The dosage was 2 gallons per animal, except herd No. 1 in 1958 which received only 1 gallon.

The insecticides used were Delnav® (2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate)) from a 30% emulsifiable concentrate, ronnel from a 25% wettable powder, Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate, also known as Bayer 21/199) from a 25% wettable powder, Bayer 22408 (*O,O*-diethyl *O*-naphthalimidophosphorothioate) from a 6.5% emulsifiable concentrate in 1957 and from a 50% wettable powder in 1958, Sevin® (1-napthyl-*N*-methyl carbamate) from a 50% wettable powder, malathion from a 57% emulsifiable concentrate, and toxaphene from a 50% emulsifiable concentrate.

After treatment all cattle were placed in the same pastures to insure equal infestation pressures.

In 1957 four herds were sprayed during the period December 13 to 19. Cattle were examined 1 day after treatment and then at weekly intervals for 3 weeks. The results are summarized in table 1. All treatments reduced populations of ticks in 1 day. Delnav, ronnel, malathion, Co-Ral, Bayer 22408, and toxaphene afforded almost 100% control after 1 week and numbers of adults remained low at subsequent examinations. Sevin reduced populations, but was not as effective as toxaphene. Malathion at 0.1% and 0.25% failed to prevent reinfestation by adults emerging from nymphal skins.

In 1958 two herds were sprayed during the period December 12 to 16. Cattle were examined after 1 week and then at monthly intervals for 4 months. Results are summarized in table 2. In herd 1 all treatments appeared to afford equal control throughout 3 months. The cattle in herd 2 were heavily infested with ticks, and all treatments reduced populations in 1 week. After 1 week and 1 month all insecticides afforded slightly better control than the toxaphene standard. Two months after treatment, the numbers of ticks increased, and Delnav and Sevin were more and ronnel was less effective than the standard. After 3 months, populations on all groups had not increased above those observed the previous month.

After 4 months populations of ticks in both herds had declined considerably, indicating the end of the winter tick season.

Table 1.—Effect of insecticide sprays on populations of winter ticks on cattle, 1957.

Insecticide	Concentration (%)	Number of Adults on 5 Animals: Before Treatment	After: 1 Day	After: 1 Week	After: 2 Weeks	After: 3 Weeks
Herd 1						
Co-Ral	0.5	15.8	4.4	0	0	0
Malathion	.25	10.6	6.2	12.4	8.8	5.0
Sevin	.25	20.8	10.6	3.8	6.0	5.6
Toxaphene	.5	18.4	3.2	0.8	1.8	2.4
Herd 2						
Bayer 22408	0.25	16.4	4.6	0.2	1.0	1.0
Malathion	.1	11.8	5.4	10.0	12.2	13.6
Sevin	.1	12.8	3.0	2.2	5.0	2.0
Toxaphene	.5	17.0	1.8	0	1.2	0
Herd 3						
Delnav	0.15	6.6	2.0	0.2	0	0.2
Malathion	.5	10.2	2.4	0.8	0.8	1.2
Ronnel	.5	9.2	5.0	0.8	0.2	1.0
Toxaphene	.5	7.6	0.4	0	0	0
Herd 4						
Co-Ral	0.25	10.0	0	0	0	0.2
Ronnel	.25	9.8	0.8	0.8	0.8	0.2
Toxaphene	.5	8.0	1.2	0	0	0

Table 2.—Effect of insecticide sprays on populations of winter ticks on cattle, 1958.

Insecticide	Concentration (%)	Number of Adults on 5 Animals: Before Treatment	After: 1 Week	After: 1 Month	After: 2 Months	After: 3 Months	After: 4 Months
Herd 1							
Delnav	0.15	8.8	1.8	2.2	3.2	1.8	0
Co-Ral	.5	8.0	0	0.6	1.2	1.2	0
Ronnel	.5	9.2	2.2	1.0	2.8	2.2	0.2
Toxaphene	.5	8.8	0.2	1.2	2.0	1.0	0
Herd 2							
Delnav	0.15	89.4	2.2	0.4	3.6	8.2	0
Sevin	.5	84.8	0.8	1.4	8.8	17.2	0
Bayer 22408	.5	90.8	0.6	2.8	11.2	13.8	0
Ronnel	.5	130.2	2.8	2.0	17.4	12.6	0
Co-Ral	.5	53.4	0	0.4	12.0	7.2	0.6
Toxaphene	.5	95.2	4.4	4.2	14.8	19.0	0.4

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Cattle Grubs in Gullets of Cattle from Southwestern Texas[1]

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In the United States the seasonal occurrence of the different stages of the common cattle grub, *Hypoderma lineatum* (De Vill.) and the northern cattle grub, *H. bovis* (L.), varies according to the latitude. To insure control of first-instar larvae with systemic insecticides, these compounds must be administered after heel fly activity has ceased and before larvae appear in the backs of cattle. During this period first-instar larvae of *lineatum* are located in the submucosal tissues of the esophagus. In any area, information on the abundance of larvae in these tissues would aid in determining the proper time of treatment with systemic insecticides.

Around Dallas, Texas, larvae were found in esophagi at all times of the year, with peak populations in September and the fewest in December–April; in New York State none were found in April–June, and the peak was recorded in October (Bishopp *et al.* 1926). In South Dakota none were found in May and June, and the highest populations were recorded in September–November (Lee & Rogoff 1954, Lee *et al.* 1955). In Oregon no grubs were present in March–June and highest populations in October and December (Roth 1957).

For a period of 2 years gullets were collected at weekly or semiweekly intervals from locker plants at Ingram and Kerrville, Texas, and taken to the laboratory and examined for *lineatum* larvae. Most of the gullets came from eight counties—

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[2] Bobby Moore and T. M. Whetstone aided in this research.

Table 1.—Incidence of grubs in gullets of cattle from southwestern Texas, 1957–59.

Month	Gullets Examined	Per Cent Infested	Average Number of Larvae per Gullet	
			Total	Infested
		1957		
May	91	68.1	4.7	6.9
June	118	78.0	5.0	6.4
July	162	79.0	6.4	8.1
August	125	81.6	7.5	9.2
September	90	66.7	6.2	9.3
October	89	59.6	2.5	4.2
November	43	25.6	0.5	1.9
December	61	4.9	0.1	1.3
		1958		
January	82	0	0	0
February	90	0	0	0
March	101	14.8	0.5	3.7
April	110	27.3	0.7	2.7
May	100	57.0	3.4	6.0
June	130	64.6	4.0	6.2
July	138	71.0	4.6	6.5
August	148	58.1	4.0	6.9
September	104	72.1	6.1	8.5
October	82	58.8	4.2	7.1
November	96	40.6	2.2	5.5
December	79	13.9	0.4	2.6
		1959		
January	78	0	0	0
February	90	0	0	0
March	110	0	0	0
April	108	19.4	0.4	2.1
May	108	30.5	1.1	3.5

Bandera, Edwards, Gillespie, Kerr, Kimble, Mason, Menard, and Real—located on or bordering the Edwards Plateau in southwestern Texas. The findings are pooled according to months in table 1. The percentage of gullets infested and numbers of grubs per total gullets or infested gullets fluctuated in a similar cyclical fashion. From May through October more than half the gullets were infested, with largest percentages of infestations in July through September. The largest numbers of grubs per gullet, total or infested, were found in August and September. After October incidence of infestations and numbers of grubs declined rapidly until none were found in January and February, 1958, and January through March 1959. In 1958 the first larva was found during the week of March 7; in 1959 it was more than a month later. In general, peaks of infestation and numbers of grubs were later in 1958–59 than in 1957–58.

The same variation noted in the gullets was also apparent in first appearance and peak populations of grubs in animals' backs. In 1957 first grubs were found as early as the middle of August and the largest numbers in November; in 1958 first grubs were found in the middle of September and peak populations in December. Season of grub encystment for 1958 was considerably later than normal for this area.

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Continuous Individual Feeding of Systemic Insecticides for Cattle Grub Control[1]

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Insecticides exhibiting systemic action against cattle grubs, *Hypoderma bovis* (L.) and *H. lineatum* (De Vill.) in their migrating stages have been tested extensively during the last 4 years. Work on various aspects of this problem has been in progress at the South Dakota Agricultural Experiment Station throughout this period (Rogoff & Kohler 1959). The work reported here was done in the 1955–56 and 1956–57 grub seasons, wherein an attempt was made to evaluate the administration of a chemical in the grain ration of cattle fed in a dry lot. This mode of administration appeared to be a practical method of dosing cattle for grubs.

Materials and Methods.—In the 1955–56 tests each calf was fed a candidate chemical that had shown promise in earlier *in vitro* tests on oesophageal stage larvae (Rogoff 1955). The materials used were nicarbazin (a complex of: 4,4′-dinitrocarbanilide and 2-hydroxy-4,6-dimethylpyrimidine), S.D. 75 (2-(p-nitrobenzamido)-5-nitrothiozole), and S.D. 51 (9-aminoacridine HCl) (marked as Monacrin®). The chemicals were incorporated in the grain rations of the calves. A low dosage for about 2 months was made available as soon after weaning as practicable. Treatment began about a month prior to the normal time of the appearance of grubs. Dosages were based on average initial weights of the calves.

The calves used in this study originated in an area of South Dakota having a history of heavy grub infestations of both species. The calves were lotted according to weight and fed individually for 84 consecutive days beginning December 22. Each animal received 4 pounds of a corn and oats mixture (half and half) daily. The calves were stanchioned for the morning and afternoon grain feedings. Hay, salt, and water were available to them on a free-access basis.

The test chemicals in a dry state were mixed with corn and oats in a small mixer. This mixture was then added to a larger amount of grain to provide 1 gram of the chemical in 1 pound of the grain mixture. The grain ration of each treated calf included 1 pound of medicated feed each day.

Grub extractions were made at monthly intervals after the appearance of grubs in the backs of the calves. Periodic weight gains were observed on each calf. Results of these tests are shown in table 1.

The effectiveness of ronnel against grubs was reported in the spring of 1956 (Lindquist 1956). The 1956–57 tests (table 1) were conducted similarly to those of the previous year. Ronnel was fed at two levels, one level for 7 consecutive days and a lower level for 79 days. A procedure similar to that of 1955 was used in mixing the chemicals with the feed and in the feeding method except for the higher level of ronnel in which the ronnel used was a 50% emulsifiable formulation, suitable for internal administration. At one of the daily feedings, the formulation containing the ronnel was poured on the feed and mixed. Vials

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[2] Associate Animal Husbandman, Entomologist, and Assistant Animal Husbandman, respectively, in the South Dakota Agricultural Experiment Station. Some of the data included herein were submitted by the senior author to the Graduate Faculty of the University of Minnesota in partial fulfillment of the requirements for the Ph.D. degree.

Table 1.—Infestation and weight gain of calves fed candidate systemic agents in grain for the control of cattle grubs.

CANDIDATE SYSTEMIC AGENT	TREATMENT				NO. OF CALVES	GRUBS/CALF		CONTROL (%)	AVERAGE DAILY GAIN (LB.)
	Grams/ Day	Mg./Kg. (Initial)	Date (Initial)	No. of Days		Range	Average		
				1955–56					
Nicarbazin	1	5	12/22	84	3	15– 52	37.3	36	1.75
S.D. 75	1	5	12/22	84	3	38– 74	42.3	23	1.72
S.D. 51	1	5	12/22	84	3	22– 81	53.3	3	1.84
Control	—	—	—	—	3	41– 80	55.3	—	1.89
				1956–57					
Nicarbazin	5	25	12/6	91	3	3–101	40.3	48.5	1.16
Ronnel	3	15	12/6	77	6	0	0	100	1.19
Ronnel[a]	6	30	2/1	7	3	0– 1	0.3	99.6	1.26
Control	—	—	—	—	8	19–147	78.3	—	1.48

[a] Average grub count 4.7 per calf prior to treatment.

containing an individual daily dosage were prepared before the feeding period began. Nicarbazin was fed at five times the dosage used the previous year and for a longer period.

Some difficulty was experienced in getting the calves to consume the treated feed in the 1956–57 tests, but after a few days the medicated feed was consumed readily. The apparent palatability of the mixture was not lowered when ronnel was fed at the higher level.

DISCUSSION.—Despite the *in vitro* promise of the materials used, the 1955–56 tests were negative. Calves in all treatments were heavily infested with grubs. Analysis of variance (Snedecor 1957) revealed no significant differences between lots when comparing either average numbers of grubs or average daily gains in weight.

In the 1956–57 tests, nicarbazin, fed at about five times the dosage of the previous year, produced only slightly greater grub reductions than in the earlier tests. The feeding of ronnel in the grain mixture provided outstanding reductions. No symptoms of toxicity were observed in any of the calves. The average daily gain of the untreated calves appeared higher than for the treated calves, but the differences were not significant.

The impressive results shown here of administering ronnel in feed for grub control are supported by the related work of other investigators. Sturdy (1958) reported complete control at a dosage as low as 4.25 mg./kg. for up to 7 months. Weintraub *et al.* (1959) obtained 94% control at a dosage of 10 mg./kg. fed individually for 18 days. Knapp *et al.* (1958) reported increases in grub encystments of 0.25 grubs per animal in the treated animals and 3.2 grubs per animal in the untreated ones after ronnel was fed in a pelleted feed for 1 day at 110 mg./kg. per animal. Weight gain differences between treatments were inconsistent and not caused by grub treatment alone. DeFoliart *et al.* (1958) fed ronnel to calves for 2 days at an average dosage of 55 mg./kg. Sixty-one per cent fewer *H. lineatum* and 78% fewer *H. bovis* encysted in the treated calves compared with their controls. These investigators, with the exception of Weintraub *et al.* (1959), offered the medicated feed in bunks, cafeteria-style. Calves fed individually as described in the present paper show a more consistent grub reduction. It appears that if calves are to be fed a systemic against grubs, cafeteria-style, they should all be eating regularly before the chemical is added to the feed to help achieve uniform consumption. A feeding period of 1 week at 30 mg./kg. per calf per day would use more chemical than the 1- or 2-day periods, but an effective dosage for each animal may be more reliably achieved. Further study on this point is warranted.

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Studies with Phenothiazine and Hypolin for the Control of Cattle Grubs[1]

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The possibility of using phenothiazine as a systemic agent against cattle grubs (*Hypoderma spp.*) has been under consideration for many years. Some investigators have noted fewer grub encystments in cattle treated with anthelminthics, including phenothiazine. Early reports were contradictory but interesting. Spence (1952) reported negative results against grubs when using several periodic oral doses of phenothiazine. Worden (1953), however, reported outstanding differences in grub counts of cattle receiving repeated oral doses when compared with their untreated controls. Worden used Hypolin (a proprietary mixture of phenothiazine, di-n-butyl tin dilaurate, and hexachlorethane) as the test chemical. Schwartz *et al.* (1954) reported

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[2] Associate Animal Husbandman and Entomologist, respectively, in the South Dakota Agricultural Experiment Station. Some of the data included herein were submitted by the senior author to the graduate faculty of the University of Minnesota in partial fulfillment of the requirements for the Ph.D. degree.

reductions in grub numbers in cattle that were allowed free-choice access to phenothiazine in mineral and grain. These conflicting reports and the interest generated by them prompted these studies.

MATERIALS AND METHODS.—Tests were initiated at the South Dakota Agricultural Experiment Station in December of 1954 to test further the efficacy of phenothiazine and Hypolin against cattle grubs. Hypolin was administered as a drench to four calves at 250 mg./kg. body weight. Grub extractions showed these calves had an average of 59.5 grubs compared with 63.0 grubs for the control lot, indicating no effect on grub encystments (Rogoff 1955).

In 1955 yard tests at Brookings, S. D. 119 (di-n-butyl tin dilaurate), a constituent of Hypolin, was administered as a drench to three calves. Each calf received 2 grams per month for 4 consecutive months beginning in December. The treated calves averaged 55.6 grubs per animal compared with 55.3 for the untreated. No grub reduction was apparent (Rogoff & Kohler 1956).

One hundred twenty head of Angus calves were used as test animals in field studies in 1955. The calves were of similar breeding and came from an area within a county that appeared to have a uniform grub infestation. The calves were divided equally among three substations of the South Dakota Agricultural Experiment Station. Each of the substations, which are located near Eureka, Cottonwood, and Highmore, South Dakota, respectively, received 4 lots of 10 head each. The calves were lotted for a nutrition study on the basis of weight and origin. For the grub treatment, five calves in each lot were drenched with a test compound and the remaining five were untreated and served as controls. At the Highmore Substation all the animals were treated for grubs in order not to interfere with the nutrition study. The control animals for this station came from the other substations.

At the Eureka Substation, 20 calves were drenched four times at monthly intervals beginning November 15 with 1½ ounces of phenothiazine per animal. At the Cottonwood Substation similar procedures and numbers of animals were used except that Hypolin was used as the test chemical. At the Highmore Substation phenothiazine was mixed (1–9) with trace mineralized salt and fed free choice as the calves' only source of mineral supplement. The calves consumed an average of 1.35 grams of phenothiazine per day for 144 days begininng November 22. Periodic weight gains were recorded and monthly grub counts were made.

RESULTS.—The results of grub counts of these tests are shown in table 1. The Hypolin- and phenothiazine-treated calves showed, in most cases, a grub count averaging higher than that of the controls at each of the three monthly counts. This condition indicated that no grub control was achieved. In the phenothiazine-salt treated calves, the grub counts were lower than the control animals at the other two substations. The grub counts in this test show an average of 11.8 grubs per animal and an infestation of 92%. This indicates small, if any, grub reductions. Weight gains were not significantly affected by the treatments.

Fecal egg counts on the same calves by Hugghins (1957) indicated that these calves were heavily parasitized by *Moniezia*, *Nemetodirus*, and *Strongyloides*. The phenothiazine, as might be expected, had little effect on these helminths.

These results are in agreement with the early study reported by Spence and with other work conducted just prior to and after the tests at this station. Cobbett *et al.* (1957) reported that 2 to 4 grams of phenothiazine incorporated in feed did not appear to reduce grub numbers, and observed that cattle treated by drench with the constituents of Hypolin did not affect grub numbers or numbers of cattle infested. Riedel *et al.* (1955) and Roberts *et al.* (1957) reported no apparent effect on grub encystments in cattle that were fed phenothiazine at low levels in feed during intervals extending up to a year.

The data presented in this study provide no support for the use of phenothiazine or of Hypolin for the control of cattle grubs.

Table 1.—Grub counts of calves drenched with phenothiazine, Hypolin, or allowed free-choice access to phenothiazine in trace mineralized salt.[a]

	Substation and Treatments				
	Eureka		Cottonwood		Highmore
Lot No.	Phenothiazine[b] (Drench)	Control	Hypolin[c] (Drench)	Control	Phenothiazine[d] (in Salt)
I					
No. of animals	5	5	5	5	9
Grubs/head	21.8	24.8	30.4	4.4	10.7
II					
No. of animals	5	5	4	5	9
Grubs/head	36.4	33.4	25.5	23.2	14.7
III					
No. of animals	5	5	5	4	10
Grubs/head	31.6	18.0	12.0	6.5	13.3
IV					
No. of animals	5	5	5	5	10
Grubs/head	34.0	15.2	27.6	15.4	8.7
All lots					
No. of animals	20	20	19	19	38
Grubs/head	30.9	22.8	23.8	12.7	11.8

[a] Treatment for grubs superimposed on nutrition study. Grub data are the sum of three monthly counts beginning in February.

[b] Phenothiazine: 1½ oz./head/month (equiv. to 210 mg./kg., first treatment) for 4 months, starting Nov. 16, 1955.

[c] Hypolin: 1½ oz./head/month (equiv. to 210 mg./kg., first treatment) for 4 months, starting Nov. 22, 1955.

[d] Phenothiazine mixed 1:9 in trace mineralized salt: average 1.35 grams/calf/day (equiv. to 7 mg./kg., initial weight) for 144 days beginning Nov. 22, 1955.

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A Storage Rack for Insect Specimens Preserved in Liquids[1]

RALPH A. KILLOUGH, *Department of Entomology, Purdue University, Lafayette, Indiana*

Storage of insect specimens preserved in liquids has always created a problem. Designs of several different racks were presented by Peterson (1955) and Evans (1958). A rack is needed that can be constructed at a low cost, will hold a number of vials, provide easy access to the vials, and permit examination of labels without removing the vials. It is believed that the storage rack described here (figs. 1 and 2) meets all of these requirements. The Department of Entomology, Purdue University, has found this storage rack successful for storing insect specimens that are preserved in liquids (Chandler 1959).

The rack was designed to hold 144 two-dram vials or 138 three-dram vials. A standard $18'' \times 36'' \times 77\frac{1}{2}''$ (outside dimensions) metal cabinet will hold 70 racks and therefore will serve as storage for 10,080 two-dram vials (144 vials per rack, 14 racks per shelf, 5 shelves per metal case). A wooden strip, $1\frac{3}{4}'' \times \frac{1}{4} \times 24'$ provides enough material to construct one rack. The total cost of material for a rack was slightly under $1.50; this will vary with the local price of the wood. The author has cut and assembled as many as three racks per hour.

One of the main advantages of the rack is the ease with which a person can handle the large number of vials at one time. Figures 1 and 2 show that the labeling and contents of a vial may be observed without removing the vial from the rack. When one wishes to remove a vial from the rack, it may be done easily by pushing the vial upward and pulling the bottom of the vial outward (fig. 1, end view). The base of the rack is large enough to prevent it from being easily tipped. If the rack is tipped over, the strips at the top and bottom (parts B, C, and D) prevent the vials from falling out.

In order to locate specimens quickly, a label (part G) may be placed on the end of the rack giving information on the specimens in the rack. Also, a wooden dowel (part H), the size of a vial may be placed in the rack to indicate the different species. The rack can be converted easily into a vial holder for field work by attaching a handle to the top and hinging a $1\frac{3}{4}'' \times \frac{1}{4}'' \times 17\frac{5}{8}''$ strip on the part D; this will prevent vials from falling out if the rack is not completely filled.

The dimensions of the parts are as follows:

Part	*Dimensions*
A	$\frac{1}{4}'' \times 1\frac{3}{4}'' \times 12''$
B	$\frac{1}{4}'' \times \frac{3}{4}'' \times 17\frac{5}{8}''$
C	$\frac{1}{4}'' \times 1\frac{3}{4}'' \times 17\frac{5}{8}''$
D	$\frac{1}{4}'' \times 1\frac{1}{4}'' \times 17\frac{5}{8}''$
E & F	$\frac{1}{4}'' \times 1\frac{3}{4}'' \times 17\frac{5}{8}''$
G	Diptera Culicidae Aedes spp.
H	A e d e s v e x a n s

Accepted for publication June 25, 1959.

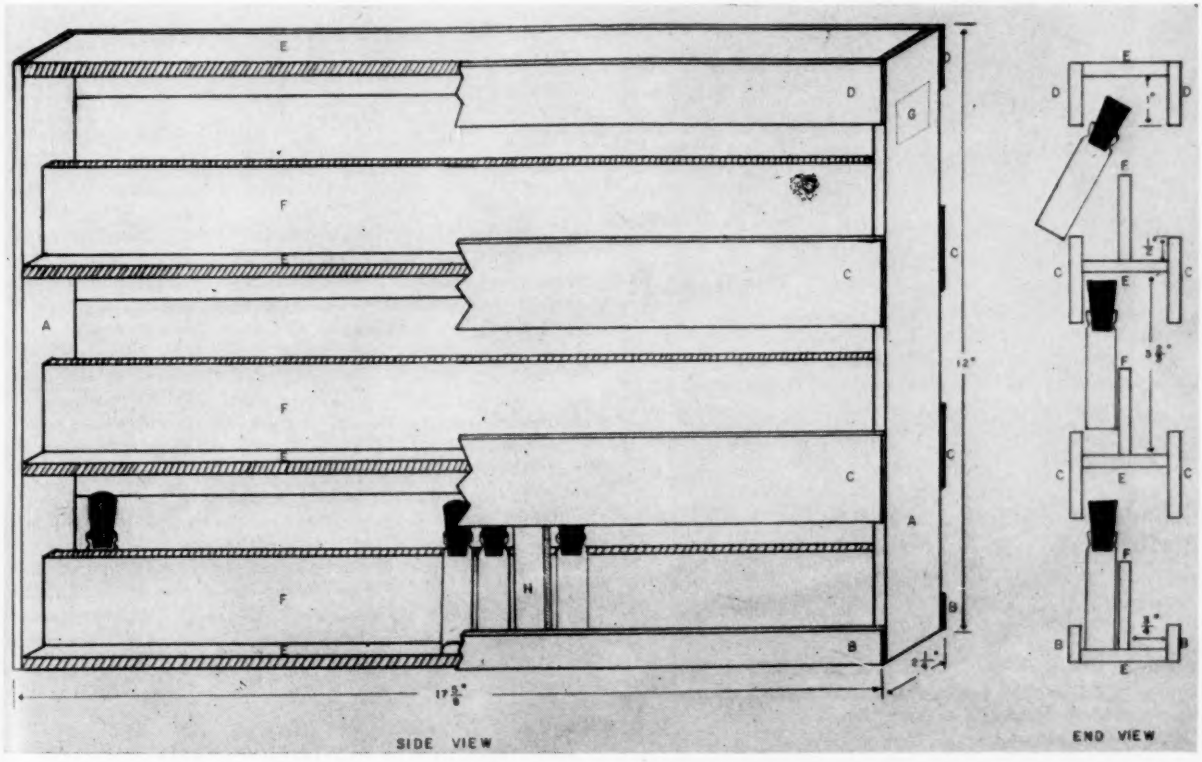

FIG. 1.—Detailed construction of vial storage rack.

FIG. 2.—View of rack in use.

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Laboratory Studies with *Bacillus thuringiensis* Berliner and Its Possible Use for Control of *Plodia interpunctella* (Hbn.)[1]

BENJAMIN H. KANTACK, *Department of Entomology, University of Nebraska, Lincoln*

The Indian-meal moth, *Plodia interpunctella* (Hbn.), is a serious pest of stored grain and cereal products and is often encountered in Nebraska in both stored wheat and corn. In many elevators and storage bins, populations of this insect develop annually and control measures are required. Infestations developing in bins of wheat and shelled corn are usually limited to the surface areas and the exposed grain mass. When unshelled corn is infested, the infestation is often found throughout the entire grain mass. In addition to attacking stored wheat and corn, Metcalf *et al.* (1951) report that this insect feeds on dried fruits, nuts, seeds, roots, herbs, and many other products.

Control measures directed against this insect have included the use of aerosols and residual sprays applied to the head space, walls and grain surface. In many instances the infested commodities are fumigated. In all cases such chemical treatments must be limited to materials that create no toxic residues. If an effective biological control agent could be developed the chemical residue problem would be eliminated.

Steinhaus & Bell (1953) tested a number of microorganisms against stored-grain insects, including *Bacillus thuringiensis* Berliner, a gram-positive spore-forming bacterium originally isolated by Berliner in 1911 from a diseased larvae of the Mediterranean flour moth, *Anagasta kühniella* (Zell.). Of the organisms investigated, *B. thuringiensis* showed significant pathogenicity to *Sitophilus granarius* (L.) and *S. oryza* (L.), both coleopterous stored-grain insects. These authors suggested fur-

[1] Published with the approval of the Director as Paper No. 985 Journal Series, Nebraska Agricultural Experiment Station. Accepted for publication September 4, 1959.

ther studies with this organism for possible control of stored-grain insects, especially the lepidopterous insects encountered in stored grain. In this same study the Angoumois grain moth (*Sitotroga cerealella* (Oliv.)) showed susceptibility to *B. thuringiensis* and, according to Steinhaus & Bell (1953), Berliner (1927) suggested that the spores of this bacillus be incorporated into the grain prior to the milling process in order to control the Mediterranean flour moth. Steinhaus (1951) found that this organism was capable of reducing a destructive population of alfalfa caterpillars in the field to a point where no economic damage occurred.

Preliminary laboratory tests showed that the larvae of the Indian-meal moth were very susceptible when *B. thuringiensis* spores were incorporated in the culture media. The present test was designed to determine what effect this material would have when applied to whole grain.

METHODS AND MATERIALS.—Pawnee wheat and field run yellow dent corn, adjusted to 15% moisture, were treated with *B. thuringiensis*[2] spores at rates as shown in table 1. All treatments were applied in distilled water at the rate of 5 ml. of the spore suspension per 250 grams of grain. The untreated checks were treated with 5 ml. of distilled water. The grain was then placed in a quart jar and rotated mechanically to obtain uniform coverage. Each treatment consisted of 1250 grams of grain divided into five replicates of 250 grams each. Into each replicate 20 Indian-meal moth larvae 10 to 14 days old were introduced. The jars were then covered with filter paper and held at a constant temperature and humidity of 80° F. ±5° F. and 60% ±5%, respectively. Adult emergence was recorded every other day until no further emergence was observed.

Observations showed that the larvae of this insect were very susceptible to infection by *B. thuringiensis* after feeding on the treated grain for only a few hours. After acquiring the initial infection the larvae appeared somewhat sluggish and apparently did little additional feeding. The infected larvae then started to take on a discoloration as infection progressed and became somewhat shrunken in appearance. After death the larvae turned dark brown to black in color and appeared mummified.

As indicated in table 1, the adult emergence was reduced considerably by the various levels of treatment. Analysis of the data showed that the reduction in emergence was significant at the 1% level. Since the larvae were approaching maturity, the time in which they could acquire infection was limited. Further tests are planned using younger larvae. It may be possible that a lower dosage level might prove effective when the exposure period is increased. Limited observations with adults emerging from the various treatments indicated that eggs from these adults, if surface contaminated with the bacterium on oviposition, did not contain sufficient numbers of spores to prevent development of hatching larvae when placed in uncontaminated culture media. Further studies with *B. thuringiensis* may show that field control of this insect is possible.

Table 1.—Adult emergence when 10- to 14-day-old Indian meal moth larvae were exposed to grain treated with *Bacillus thuringiensis* Berliner.[3]

TREATMENT	RATE OF APPLICATION (SPORES/GM. OF GRAIN)	ADULT EMERGENCE (%)
	Wheat	
A	12,500,000	1.0
B	2,500,000	27.0
C	1,500,000	44.0
D	30,000	63.0
E	Untreated	84.0
	Corn	
A	12,500,000	12.0
B	2,500,000	32.0
C	1,500,000	25.0
D	30,000	19.0
E	Untreated	77.0

[3] F values—wheat, 12.2; corn, 16.9. Both significant at the 1% level.

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[2] Bacillus spores were furnished by the Rohm & Haas Co., Philadelphia, Pennsylvania, and the Stauffer Chemical Co., New York, N. Y.

An American Cockroach Rearing Cage[1]

E. C. CUMMINGS and J. J. MENN, *Stauffer Chemical Company, Mountain View, California*

In rearing large numbers of American cockroaches (*Periplaneta americana* (L.)) we found it necessary to construct a cage which would provide for maintenance of large numbers of mature cockroaches and permit rapid and easy collection of oothecae at regular intervals.

An improved cage is described here. The metal cage as a unit is shown in figure 1.[2] The assembly of the water and feeding devise is shown in figure 2. The cage is constructed of sheet metal having $\frac{1}{8}$" mesh metal screen walls. The bottom panel is made of $\frac{1}{4}$" mesh screen to allow the oothecae to fall through the screen. The oothecae are collected in a pan partially filled with sawdust and attached to the bottom tray of the cage. The pan is easily

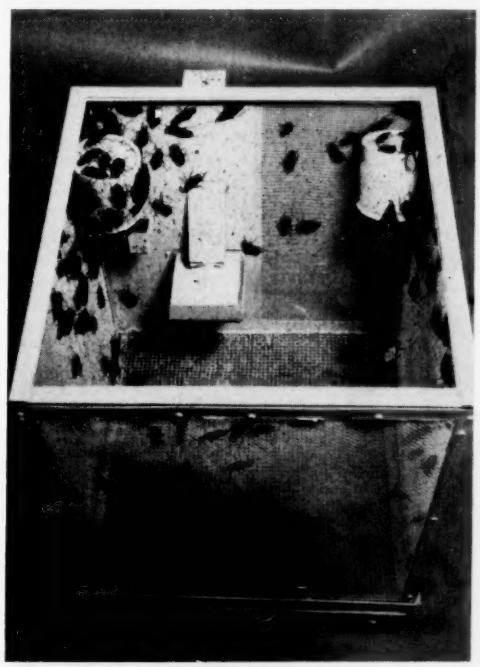


FIG. 1.—Top view of adult cockroach cage.

[1] Accepted for publication July 9, 1959.

[2] R. C. Curtis of Stauffer Chemical Company, Agricultural Research Laboratory, took the pictures of the cage shown in figures 1 and 2.

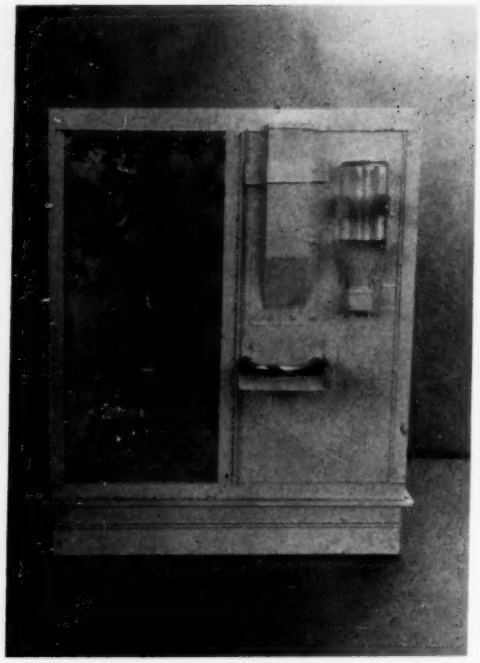


FIG. 2.—Rear view of cockroach cage, showing watering and feeding device.

removed during collection of egg cases at weekly intervals. Young mature cockroaches are added to the cage via the food drawer. Dead cockroaches are removed from the cage by pulling out the bottom panel and letting them drop into the bottom pan.

The essential parts of the cage assembly are shown in figure 3,[3] and are described below.

A. Food tray—4″×4″×1″.
B. Food tray drawer, $4\frac{1}{16}$″×$4\frac{1}{16}$″×1″, front and back sides are $1\frac{1}{4}$″ high.

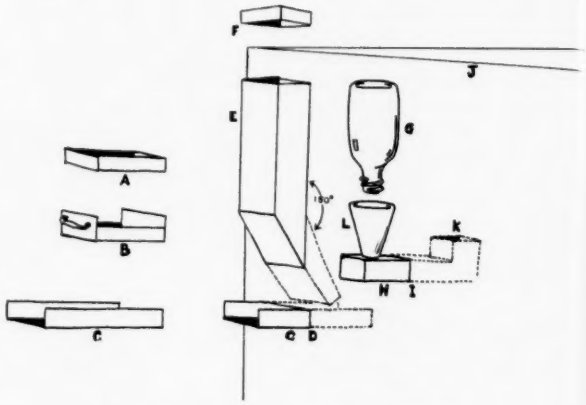


FIG. 3.—Detail of water and feeding device.

C. Food tray drawer track, 8″×$4\frac{5}{16}$″×1″, open ends.
D. Opening in back panel of cage for track (C).
E. Feeder, 2″×2″×6″, the tapered portion is 3″ long and $\frac{1}{2}$″ wide at the lower opening.
F. Feeder cover, 2″×2″×$\frac{1}{4}$″.
G. Watering bottle, 8-oz. capacity.
H. Water dispenser body, 1″×1″.
I. Opening in back plate (J) for water dispenser.
J. Back panel of cage.
K. Water dispenser, $1\frac{1}{2}$″ high, opening 1″×1″.
L. Metal cone to support water bottle.

Overall cage dimensions are 18″×18″×18″ with a 1″ flange over the bottom pan on the sides and back panel of the cage.

This cage has been adapted in several other laboratories which have found it very useful in mass rearing of American cockroaches.

[3] T. C. Allen, Jr., of the above laboratory, made the drawing of the cage details.

BOOK REVIEW

INDEX XVI TO THE LITERATURE OF AMERICAN ECONOMIC ENTOMOLOGY, JANUARY 1, 1956–DECEMBER 31, 1957. Compiled by Helene G. Cushman, Library, U. S. Department of Agriculture. 8vo., cloth, 694 pp., Washington, D. C. Entomological Society of America. Special Publication 16. 1959. Price $6.00.

In view of the fact that this reference work covers in minute detail a field of research not fully or adequately covered elsewhere, it would be a matter of difficulty to attempt to evaluate its great—its outstanding usefulness to research workers within the scope of its subject matter. It will be welcomed not only in entomological research and control operations, but also in the field of medical entomology as well, for the reason that it now completes and brings down to date an exceedingly valuable series that reaches back for 99 years and covers American economic literature from 1860 down to the present time. It may be remembered that it was the urgency of the need for a work of this kind that prompted the beginning of such compilation by B. Pickman Mann and Samuel Henshaw, and later continued by Nathan Banks. It consisted of eight volumes and covered the years 1860 to 1905, and was published under authorization of the U. S. Congress. The 15 volumes subsequent to that period were prepared by Nathan Banks, Mabel Colcord and Ina L. Hawes, assisted at various times by other interested workers. Beginning with Index VII, it became necessary to enlarge the geographical scope of the series to comprise continental North America, including Canada, Alaska, Mexico, Panama, Canal Zone, Cuba, Puerto Rico, Hawaii, and certain other Pacific Islands, particularly those that played a part on World War II. In all of these, particular attention was given to keeping up with most recent periodical literature dealing with such subjects as the newer insecticides, and the latest approved methods for their application, and, especially, latest results obtained from tests of new compounds and the like. Indeed so very rapid have been developments along some of these lines that it becomes of considerable interest merely to note from one of these something of the extent to which investigations have been broadened from the previous volume in the use of new words, representing names of new compounds, new subject headings, or new types of equipment. These additions to the vocabulary represent definite milestones in the advancement of our knowledge. Because of the growing multiplicity of cross references to chemicals and their duplication in the various chemical sections of the Index, such references in volumes XIV, XV and XVI have been removed from the text and placed as separate units at the end of the respective volumes, with appropriate footnotes to refer the student to such cross reference lists. On the whole, this book represents an exceedingly helpful addition to a series that is growing in value with each additional volume. The compilation of a bibliographical assignment of such wide scope as this entails sustained interest, and enormous labor over prolonged periods of time in its preparation. Grateful thanks from all who make use of this book are due to Mrs. Cushman and her staff for its surpassing excellence.

J. S. WADE

OBITUARIES

Willis Everett Snow
1918-1959

Willis Everett Snow died in London, June 12, 1959. He was 40 years of age. Dr. Snow was returning to the States to resume his work with the Tennessee Valley Authority after completing an entomological research project at the East African Virus Research Institute, Entebbe, Uganda. The research project had been made possible through a Fulbright Scholarship. The exact cause of Dr. Snow's death has not been determined, but it was ascribed by the coroner to natural causes. "Willie" was widely known as an enthusiastic and energetic worker. These characteristics had led him to work very hard and for long hours on his project, and his associates observed that he was quite tired when he left Africa.

Dr. Snow was born December 8, 1918, at Chicago, Illinois, and attended high school at Oak Park, Illinois. He studied entomology and botany at the University of Illinois and received his A.B., M.S., and Ph.D. degrees in 1942, 1944, and 1949, respectively. While at the University he was Chairman of the Campus Guide and Travel Committee. He spent considerable time in Boy Scout work.

His professional experience included work as a pest control operator, as an entomologist with the U. S. Public Health Service engaged in malaria control work in war areas, and as teaching assistant at the University of Illinois and at The Principia, Elsah, Illinois. For the past 10 years his outstanding ability and energy had been principally devoted to technical field and laboratory studies on the biology and ecology of the immature and adult stages of mosquitoes and associated organisms as related to the reservoirs of the Tennessee Valley Authority. For many years he was also actively interested in the ecology of microcosms such as might be found in tree cavities and other plants. His doctoral dissertation, "Arthropods of Wet Tree Holes," is gaining fame as a classic in the field. In pursuit of this interest, he obtained a grant from the Philosophical Society of America and studied the maguey plant in Mexico. As a Fulbright scholar, he was studying the fauna of tree holes and bush-baby nests, as well as the swarming and biting habits of mosquitoes and other Diptera in the dawn and sunset periods. His interest in insects as components of an environment made him an entomologist's entomologist, and his love of field work made him an invaluable source of information about specific habitats.

During his last 2 years of work for TVA, Dr. Snow published 12 manuscripts. Three more were left incomplete. He was an active member of the Entomological Society of America, American Mosquito Control Association, and the Ecological Society of America. Through personal contacts and communications, he established mutually beneficial and harmonious working relationships with people all over the world. His contributions to entomological and ecological science will long be remembered in international scientific circles.

Dr. Snow's way of life was an inspiration to all who knew him well. Perhaps his greatest attribute was his love and respect for other people and his unselfish desire to be of service. Those who were associated with him feel keenly the loss of a close friend. The profession has lost a highly competent entomologist in the midst of a career already brilliant and productive.

GORDON E. SMITH

Willis Everett Snow
1918–1959

Ernest Adna Back
1880-1959

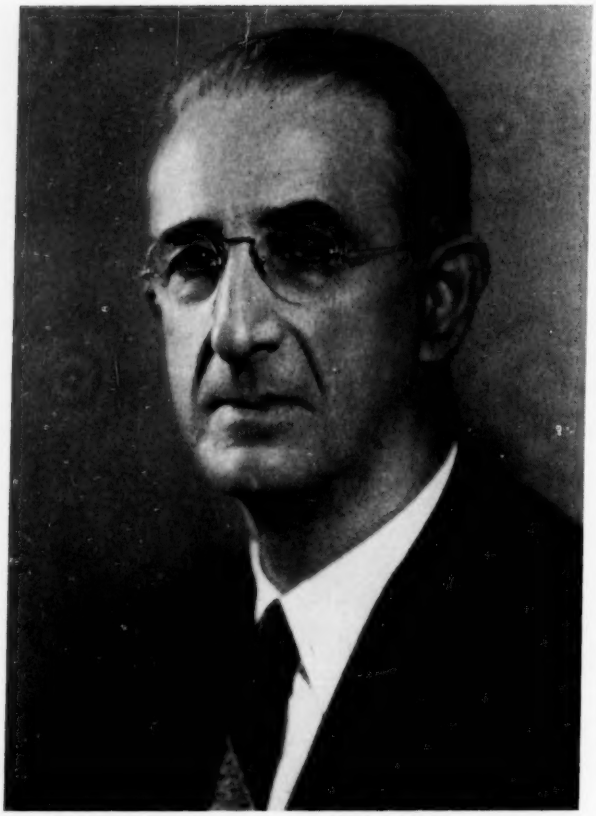
If a quiet man slowly makes many friends during his lifetime it means that there are in him qualities which, gradually revealed to his associates, bind people to him "for keeps." Such a man was Ernest A. Back. Few people called him Ernest; most of us referred to him with affection and respect as Dr. Back. Once you gained his friendship you knew that you had hold of something permanent, and that something permanent had hold of you.

Ernest Back's boyhood was not as easy as the younger days that many of us enjoyed. He was born Oct. 7, 1880 in Northampton, Massachusetts, and grew up in Florence, a village in that town. When he was only seven his father died of tuberculosis. His mother, Mary E. Young Back, passed away 2 years later. He had two devoted sisters, one a year younger and the other 10 years older. The children were brought up together in Florence by "Aunt Rosie" Harris, his father's sister. After Ernest's older sister married, he made his home with her for many years and enjoyed a brother-sister relationship with his four nieces and nephews.

Following his preparatory education in the local public schools he entered nearby Massachusetts Agricultural College, now the University of Massachusetts, in Amherst, in the fall of 1900, graduated in 1904, and received his Ph.D. degree there in 1907. His studies in entomology were guided by the Fernalds, Charles H. and Henry T. Dr. Back's thesis subject was a study of the robber flies.

During his years as a college student he earned money as Deputy State Nursery Inspector (1902–1906), instructor in entomology at MAC (1904–1906), and instructor in botany (1906–1907).

After completion of graduate work, Dr. Back was appointed to a position with the Bureau of Entomology, USDA, for work



Ernest Adna Back
1880–1959

on control of citrus whiteflies. These investigations were begun at a field laboratory in Orlando, Fla. In this laboratory he played a leading role in pioneer research on control of the whiteflies and other aleyrodid pests of citrus. These investigations were extended to Cuba and Mexico and included control measures by the use of entomogenous fungi, insecticide sprays, and fumigants. In 1910 he was appointed entomologist and plant pathologist, Virginia Agricultural Experiment Station, and State Crop Pest Commission, Virginia Polytechnic Institute, Blacksburg, Va. He filled this position with distinction until 1912 when he was recalled to service with the Bureau of Entomology for work on fruit fly investigations. For the next 5 years, 1912–1917, he carried out research on the biology and control of fruit flies in Hawaii, Bermuda, and Spain. Control measures that were developed as a result of these investigations played a major role in the control or eradication of these insects.

In 1917 Dr. Back was chosen to head the new Division of Stored Product and Household Insect Investigations in the Bureau of Entomology (later designated Bureau of Entomology and Plant Quarantine) as principal entomologist. His outstanding work in this field continued until 1934 and made fundamental contributions to our knowledge of the biology and control of pests of the household and stored products. Clothes moths, carpet beetles, pests of stored grains and milled cereals, infestations in flour mills, dried fruit insects, pests of tobacco and tobacco products, insects affecting stored vegetable products, and pests of cured meats were included in the extensive investigations. The problem of preserving stored grain on the farm from insect attack also received study and valuable control recommendations were made. As a result of basic research with fumigants, including hydrocyanic acid gas, ethylene oxide-carbon dioxide mixture, and chloropicrin, he contributed greatly improved apparatus and techniques for fumigation of commodies in warehouses, in vacuum chambers, in chambers at atmospheric pressure, and under tarpaulins. From 1934 to retirement in 1947 he served with the Division of Insects Affecting Man and Animals of the Bureau of Entomology and Plant Quarantine. After a distinguished career of 40 years in entomological research and administration, he retired in 1947. The family home in Washington was at 2936 Macomb Street, N.W., where he resided until 1951 when he moved to Chaplin, in East Central Connecticut.

Entomologists throughout the world who are interested in the insect pests of stored products do not need to be reminded of the contributions to their branch of science made by E. A. Back. More than 100 bulletins and articles, illustrated with often-copied drawings and excellent photographs, remain to his credit. His writing style was notable for its clarity.

Dr. Back's membership in scientific societies included the Entomological Society of America, Washington Academy of Sciences, Entomological Society of Washington, and the American Association for the Advancement of Science. He was a member of the Cosmos Club, Washington, D. C., for 40 years.

He died in the family home on May 21 of this year. He is survived by Mrs. Clara N. Back, Chaplin, Conn.; two sons, David N. Back, Southwick, Mass., and Dr. Richard C. Back, Irvington, N. Y.; and five granddaughters.

What a man does when he is not earning his living may be more interesting than his activities from 8 to 5 on work days. This was true of E. A. Back. Several of his hobbies were out of the ordinary. While living in Washington he collected old millstones, probably as a result of visiting country flour mills. He gathered more than two dozen of them and when he moved to Connecticut he took with him all but some of the largest which, no doubt, remain in his terraced back-yard garden close to Rock Creek Park. He was fond of antique furniture, early farm implements, clocks, pottery, and bottles, all of which he collected to a modest extent. His interest in history and genealogy was reflected in membership in the Sons of the American Revolution, the Order of Founders and Patriots, the Society of Colonial Wars, and the Mayflower Society.

Ernest Back was working in Florida when he met Clara Newcomb, a visitor to Orlando from Connecticut. They were married in September 1919. A journey across the United States, combining business and honeymoon, followed. When their sons had reached their late teens the family went to the Pacific Coast for another long journey, this time by automobile.

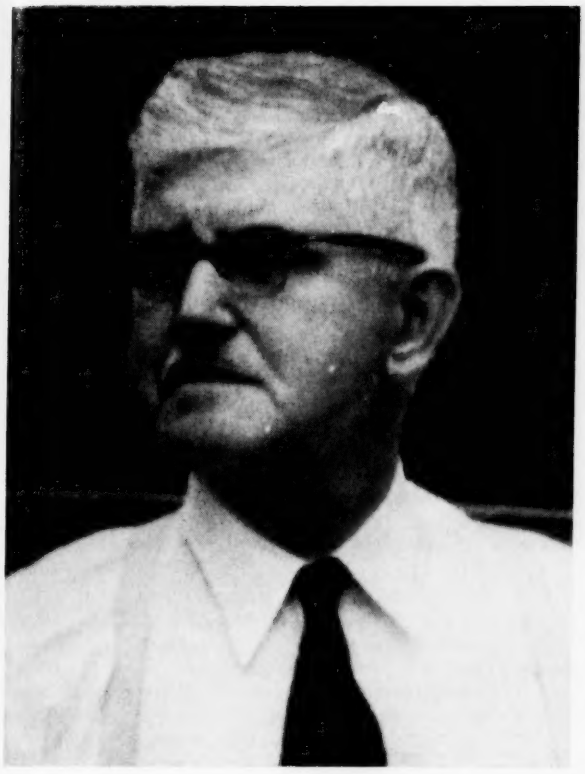
Those of us privileged to work with him on research problems or to accompany him on field trips were treated to a fine experience. His integrity, modesty, sincerity, generosity, patience, and gentle humor were qualities that endeared him to a host of co-workers and friends. He was a sensitive man, and always the courteous gentleman.

Mrs. Back will continue to live in the old village home in Chaplin and will welcome her husband's friends who pass that way.

W. DOYLE REED
PEREZ SIMMONS
AUSTIN W. MORRILL, JR.

James Zetek
1886-1959

Born on December 12, 1886, at Chicago, Illinois, James Zetek graduated in 1911 with an A.B. degree from the University of Illinois. When he was 25 he went to Panamá where from 1911 to 1913 he was entomologist for the Isthmian Canal Commission. In 1914, Jim married María Luisa Gutierrez, a member of a greatly respected family of Panama City; both were devout Catholics. Until she married, Jim's sister Ella lived with the



James Zetek
1886–1959

Zeteks, universally known as their daughter. From 1914 to 1915, Jim was employed by the Republic of Panamá as director for the organization of the National Exposition held at Panamá City from 1915 to 1916. From 1916 to 1918, he was professor of biology and hygiene at the National Institute of Panamá, and from 1918 to 1920 he held the post of entomologist for the Panama Canal.

In November, 1920, Zetek was appointed in the Bureau of Entomology as a "specialist on blackfly," a title probably used because he had collaborated for several years with federal workers in a study of the citrus blackfly, a pest of major importance in many tropical or subtropical areas. Results of his work were published in Department Bulletin 885 of which he was co-author with the late Harry F. Dietz.

Zetek's main official assignment was a study of tropical fruit flies, many species of which occur in Panamá. He was specially well-fitted to investigate the biology, habits, host fruit preferences, and especially the method of egg-laying of different species in various fruits. Out of about 126 valid species discussed by Alan Stone in U. S. Department of Agriculture, Miscellaneous Publication 439, "The Fruit Flies of the Genus *Anastrepha*," 54 were collected in Panamá by Zetek, and in a number of cases his specimens were the only ones available. Although most of his work dealt with biology of these insects, he was the first to determine that tartar emetic is an effective, although slow-acting, poison for use in bait sprays for many species of the group.

Jim also collected many species of termites and studied their habits and destructive work. When Barro Colorado Island was reserved for scientific purposes on April 17, 1923, Zetek with Snyder, began tests on the resistance of native and exotic woods to termites and to decay, both in natural state and after treatment with chemical wood preservatives. Stakes, sections of telegraph poles, bridges, a watch tower, a water tank, and several habitable buildings, serving as rest houses, located at various points on the island were used. In addition, tests were made of treated fiber board and insulated wire. Most of this material was shipped from the United States to the Canal Zone, where it was placed under test, inspected, and reported by Zetek.

When the International Termite Exposure Test was instituted in various tropical countries by George Hunt of the Forest Service and Thomas E. Snyder, Zetek, from 1928 to 1952 installed, inspected, and reported on treated lumber shipped to Barro Colorado Island. During World War II, he conducted secret deterioration studies on the island for the Department of Defense, and for the Eastman Kodak Co.

In 1923, in cooperation with the Office of the Panama Canal, he began tests at Balboa on the resistance of treated and untreated piling to attack by the various marine borers found in the ocean waters of that region. As with the termite tests, yearly inspections and reports were made.

While thus employed by the U. S. Department of Agriculture, he served from 1923 to 1945 as Curator of the Barro Colorado Island Biological Laboratory, established first as the Institute for Research in Tropical America under the National Research Council, and in 1939 given legal status by act of Congress as the Canal Zone Biological Area under a board administered by the National Academy of Sciences. When this entity was transferred to the Smithsonian Institution in 1946, Zetek became resident manager, though he continued to live at his old location at No. 0902 Amador Road in Balboa. He was transferred to Smithsonian pay rolls November 6, 1951, and continued there until his retirement on May 31, 1956. On June 1 following he was made an honorary Research Associate of the Smithsonian, a title that he held until his death.

Zetek, with Thomas Barbour of Harvard University, was one of the ardent advocates of the reservation of Barro Colorado as a wildlife preserve for scientific study. While in charge he was an ambassador of goodwill to visiting scientists from all parts of the world. It is this laboratory that remains as the outstanding monument to his devotion to studies in all fields of tropical science. In 1948 Zetek arranged for a 10-cent memorial stamp issued by the Canal Zone on the 25th anniversary of the laboratory, appropriately bearing the ubiquitous coati mundi as the design.

The appended list of publications by Jim shows his wide interests from insects and mollusks to *Peripatus*. He did much general collecting and had many new species named after him in protozoa, mollusks and insects, as well as a striking yellow and black spotted frog.

Jim was highly respected in the Republic of Panamá as well as in the Canal Zone. It gave him great pleasure in 1924 to take friends to call on the President of Panamá at the beautiful palace. Several honors were conferred on Zetek. In 1933 he was made an honorary member of the Scientific Staff, Byrd Antarctic Expedition. He received the gold medal of the National Institute of Panamá in 1915, 1916, and 1917, and the grand prize of the National Exposition in 1916. The Vasco Nuñez de Balboa honor of Panamá, and the Alfaro honor of Ecuador, were bestowed on him, as well as the Danish Galathea medal of 1950–1952.

Zetek was a member of the American Association for the Advancement of Science, the Entomological Society of America, Entomological Society of Washington, Society of Parasitology, American Medical Association, Ecological Society, Malacological Union, Illinois Academy of Science, Washington Academy of Science, Panamá Society of Agronomy, Canal Zone Medical Association, and Panamá Canal Natural History Society. He helped found the organization last mentioned, and took delight in electing his friends to membership. He also belonged to the Panamá Rotary Club (where luncheon talks were conducted in English, Spanish, and Chinese), to the beautiful Union Club of Panamá, and to the Cosmos Club of Washington, D. C.

Jim had a most alert and active mind. Throughout his official life his desk, and in fact his whole office, was stacked with projects—more than one man could complete in a lifetime. Yet

he accomplished much, and then looked about for more to do. He was deeply affected by the death of his wife María on October 5, 1945, and really never recovered from this loss. He died June 1, 1959 in Panamá City, after having been in ill health for several years.

Surviving members of his immediate family are a sister, Mrs. Ella Zetek Rhode of Guayaquil, Ecuador, a second sister Florence and a brother Charles, of Chicago.

THOMAS E. SNYDER, *Chairman*
ALEXANDER WETMORE
BENNET A. PORTER

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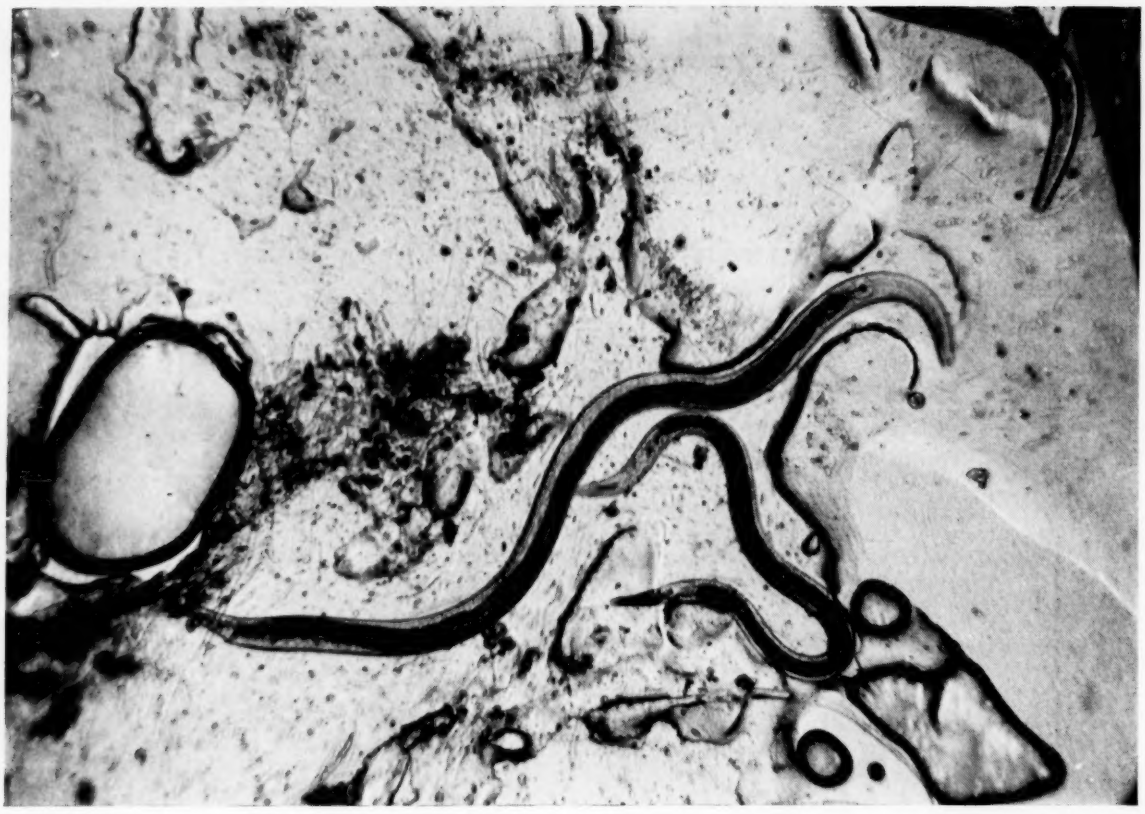

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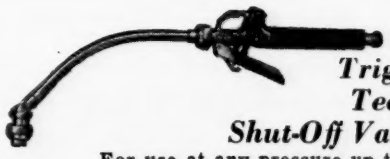

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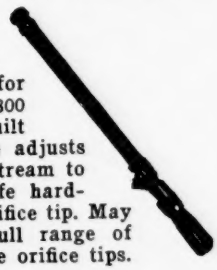

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